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BULLETIN OF THE HYGIENIC LABORATORY,

U. S. Marine-Hospital Service.

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PRELIMINARY NOTE

ON THE

Viability of the *Bacillus Pestis*

(FROM PUBLIC HEALTH REPORTS, MAY 25, 1900).

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PRELIMINARY NOTE

ON THE

VIABILITY OF THE BACILLUS PESTIS.

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United States Marine-Hospital Service.

There seem to be three factors that influence the life of the bacillus pestis in the outer world, viz, light, moisture, and temperature. The bacillus withstands quick drying very badly, as all the experiments in this direction indicate. It can not live long in the sunshine. High temperatures are invariably fatal.

That it always and under all circumstances dies in so short a time as five days, as the work of Kitasato and Wilm first indicated, must now be doubted in view of the experience of Abel, Ficker, Batzaroff, Hankin, The German Plague Commission, Germano, Giaksa, and myself.

The bacillus of plague does not exist in nature on glass cover slips, nor yet in the desiccator over concentrated sulphuric acid. With us it certainly would not be exposed to drying in our houses, and on fabrics, at temperatures of 30° and 37° C., conditions under which many of the tests to determine the viability of the organism were made.

We ought, therefore, not to apply the experience of the laboratory too literally to the life history of the plague bacillus in nature, for we can not imitate all the conditions under which the organism may exist. We may determine with fair certainty the length of time the plague bacillus may live under given conditions; and in general terms we can state whether it is a hardy organism, resistant to influences usually detrimental to bacterial life, or one that loses its virulence and dies quickly when removed from its natural habitat.

In this laboratory we have worked with the organism from 5 sources, 1 from Djiddah, 1 from Oporto, 1 from Rio de Janeiro, 1 from Bombay, and 1 from the New York quarantine case. We find that all these 5 races thrive well under the usual laboratory conditions on the ordinary media.

They can in no sense be considered tender organisms, as was at first supposed. They are much easier to cultivate than the lanceolate coccus of pneumonia or the streptococci. In fact, they resemble more closely the hardier of the hemorrhagic septicæmic group.

In this connection, Batzaroff (*a*) states: "It may be said that it is a mistake to consider the microbe of human pest to be very frail. There undoubtedly exist varieties that attenuate very quickly, and die outside the living body in a relatively short time. There are other varieties that retain their viability under similar conditions a long time, and continue to live in artificial media for months and even years without notably losing virulence. We have seen that certain of our cultures which have been kept in the laboratory without any precautions and exposed to the light for three and one-half months, still kill animals when injected hypodermatically with but slight retardation; and that regenerated 2 or 3 times they almost regain the virulence they first possessed. It is not always easy to obtain an attenuated culture of pest. It requires much time and a combination of various artifices."

On first glance over the literature on the viability of the organism there appears to be an irreconcilable difference among the various observers as to the length of time the organism will live and maintain its virulence outside the body, but on closer study it becomes apparent that these differences are due to different conditions under which the experiments were carried out, especially differences in temperature. We must not lose sight of the fact that some races of the organism are more hardy than others.

It is the experience of all observers that the bacillus can not live long outside the body when dried at a temperature of 30° C., or over, but at a temperature lower than this and under 20° C., it has been kept alive sixty and seventy-five days.

The German Plague Commission (*b*) found that the organism always lost its power of infection when dried, within eight days, in India, but after returning to Germany could be kept alive after drying twenty-eight days, at 15° to 18° C.

My own experience indicates that the organism, when dried, will die quickly if the temperature reaches 27° C. but that at 23° C. and under it may live a long time.

A series of experiments has been undertaken in this laboratory and a few of the results are given in advance showing how long the organism may live and retain its virulence when dried under various conditions.

ALBUMIN-GELATIN BALLS INFECTED WITH PLAGUE CULTURE.

A little ball of sterile absorbent cotton about the size of a pea is soaked with a few drops of a gelatin culture of plague (Djiddah) mixed with egg albumin, and exposed in a Petri dish in the photographic dark room (20° to 23° C.) and the cool chamber (17° to 19° C.).

a Batzaroff *La Pneumonie pesteuse expérimentale*. Annales de l'Institut Pasteur, May, 1899, page 391.

b Kaiserliche Gesundheitsamt, Bd. 16, Berlin 1899, page 274, *et seq.*

The little balls soon dry and the gelatin-albumin shrinks to a flaky, dry, hard mass.

From time to time one of the dried balls is taken out, planted in bouillon, and incubated. In case a growth appears the organism is tested on all the media, and on mice.

It was found that the organism lived and remained virulent for mice under these conditions for seventy-five days in the dark room at about 20° to 23° C. and for seventy-five days in the cool chamber at 17° to 19° C. Whether it may live longer will be determined from time to time and reported later on.

CRASH INFECTED WITH PLAGUE CULTURE.

In another series of experiments little squares of fabric (crash) were sterilized and inoculated with a three-day-old bouillon culture of the bacillus pestis. One set of these was allowed to dry out in Petri dishes in a dark corner of the laboratory where the temperature ranges from about 20° to 27° C., another set in the cool chamber (17° to 19° C.), and another set in the photographic dark room (20° to 23° C.). The squares were removed at intervals and planted in bouillon and in case a growth appeared it was studied for purity and pathogenicity in media and mice.

The following are some of the results :

Bouillon culture on crash.

Time.	Laboratory 20° to 27° C.,	Cool chamber, 17° to 19° C.	Dark room, 20° to 23° C.
4 days.....	+	+	+
8 days.....	—	+	+
11 days.....	—	—	—
13 days.....	+	+	+
15 days.....	—	—	+
21 days.....	—	+ Killed mouse in two days.	+ Killed mouse in two days.
26 days.....	—	—	+ Killed mouse in three days.
35 days.....	—	+	+
48 days.....	—	+ Killed mouse in three days.	+ Killed mouse in two days.

From this table it appears that the organism died after thirteen days when kept at a temperature which occasionally rose to 27° C.

At a somewhat lower temperature it remained alive and virulent forty-eight days. Whether longer, will be reported upon the completion of the work. This table indicates plainly how sensitive this particular organism is to a very slight difference of temperature.

PINE WOOD INFECTED WITH PLAGUE CULTURE.

Another series of tests was made with splinters of pine wood about the size of a match stick. They were sterilized and soaked in a three-day-old bouillon culture of plague and then placed in Petri dishes

which were kept in the laboratory (20° to 27° C.), cool chamber (17° to 19° C.), and the dark room (20° to 23° C.), with the following results:

Pieces of pine wood inoculated with bouillon culture of bacillus pestis.

Time.	Laboratory.	Cool chamber.	Dark room.	Time.	Laboratory.	Cool chamber.	Dark room.
4 days.....	+	+	+	13 days.....	—	—	—
5 days.....	—	+	—	18 days.....	—	—	—
11 days.....	—	—	+	21 days.....	—	—	—

The same culture was used to impregnate the pieces of pine wood as was used for the squares of crash in the preceding table, and these two objects thus infected were exposed to precisely the same conditions. It may therefore be assumed that the organism lives a shorter time on the one than on the other.

PAPER INFECTED WITH PLAGUE CULTURE.

Another series of tests was made with pieces of filter paper and pieces of glazed (sized) paper. This paper is cut into little squares and sterilized and impregnated in the usual way with a three-day-old bouillon culture of the organism. These pieces were placed in Petri dishes and kept in the desk in my office where the temperature ranges from 20° to 27° C. The results follow:

Plague culture dried on paper.

Time.	Filter paper.	Glazed paper.	Time.	Filter paper.	Glazed paper.
4 days.....	+	+	13 days.....	—	—
5 days.....	+	+	18 days.....	—	—
11 days.....	—	—			

On account of the importance of this subject, a summary of the literature follows:

KITASATO (a) found the organism alive after four days when dried on a glass cover slip and kept at 28° to 30° C. It grew after one to thirty-six hours, but not after four days. He used the pus of buboes from man. His experiments with serum cultures gave similar results. He was the first to study this question.

WILM (b) found the organism alive after four and one-half days on a glass slip. He exposed pure cultures at a temperature of 29° to 31° C. He could obtain no growth in bouillon after an exposure of four and one-half days. In vacuo, the bacilli were killed after an exposure of three hours.

^a Preliminary notice of the bacillus of bubonic plague, Hongkong, July 7, 1894.

^b Über die Pestepidemie in Hongkong im Jahre 1896. Hygienische Rundschau, 1897, Bd. VII, page 290.

R. ABEL (*a*) used pure cultures from bouillon, agar-agar and blood serum, or pus from the peritoneal cavity of guinea pigs, containing the bacillus pestis. These were planted on various objects—glass cover slips, silk, wool, woollen cloth, linen, and pieces of ox skin, and in sterile earth. Also pieces of the organs, liver, and spleen of animals dead of plague were dried. Approximately similar sizes were taken every day from each object. The result of these experiments showed that the manner of drying influenced the viability of the pest bacillus. No matter on what material, the result of drying at the temperature of 35° C. in the incubator, or 20° C. in vacuo over sulphuric acid, the bacillus was always found dead in two, or at most, four days. If the drying was permitted at the room temperature, 16° to 20° C. in a dark place, the bacilli remained alive much longer and the material in which they were dried markedly influenced the result.

On cover slips they remained alive six to nine days, from pus and cultures. Only once in four tests were the bacilli found alive a longer time—fourteen days—but not longer than this.

On threads of various sorts, on linen, and in pieces of organs, the bacilli were found alive after thirty days; if longer, it could not be determined, because the work was interrupted. In the longer exposures the threads planted in bouillon and kept undisturbed showed the outgrowth of isolated colonies here and there, indicating that many of the bacilli had lost their power of multiplication.

He, therefore, concluded that forced drying at a temperature over 30° C. or by substances that have a high affinity for water such as concentrated sulphuric acid, the bacillus pestis dies quickly. Slower drying at lower temperature is less harmful. However, even at room temperature the quicker drying, such as on a cover glass, is more harmful to the bacillus than the slower drying on pieces of cloth or in pieces of tissue.

“The fact that the bacillus remains alive longer when dried at 16° to 20° C. than was at first announced, teaches that in our climate (Hamburg) at least, we can not expect the organism to quickly disappear in clothing, etc.”

On *fresh skin* the bacillus was found alive after ten days. These tests were not altogether satisfactory because the sterilizing of the skin before infecting it with pest was not successful after washing and eight days' immersion in ether and alcohol. It is possible that the bacilli are alive after ten days, though not demonstrable on account of the growth of other organisms.

Further tests are being made with cultures, and the bacillus in blood and organs placed on various objects, and kept under various conditions, report on which will be submitted later.

MARTIN FICKER (*b*) tested the effect of drying and moisture alter-

a Zur Kenntniss des Pestbacillus. Centralblatt für Bakteriologie und Parasitenkunde, etc., Bd. XXI, 1897, page 497.

b Zeitschrift für Hygiene, Bd. XXXIX, 1898, page 25.

nately applied on the viability of the plague bacillus and found it to be more harmful than simple drying. He found that changing the conditions from dryness to moisture caused the death of the organism in twenty to twenty-eight hours, while the same in the desiccator lived eight or nine days.

He used twenty-four hour old cultures from agar-agar plates and made thin spreads on cover glasses by means of the platinum œse. Moisture was added twice daily. The cover glass was planted in bouillon and in case growth appeared this was plated out on gelatin.

	Alternate moisture.		Desiccator.	
	†	O	+	O
	Hours.	Hours.	Days.	Days.
Culture 24 hours.....	20	28	8	9
Do	24	36	9	11
Culture 36 hours.....	36	48	11	12

This demonstrates that cultures die more rapidly in drying when moisture and dryness are alternated.

BATZÁROFF (*a*) has been able to demonstrate that even in the dry state the pest organism remains living and virulent a long time. The organs of an animal dead of pest, as well as cultures mixed with infusorial earth, are subjected to desiccation in vacuo at "room temperature."

From time to time a piece of the dried substance is tested by grinding in a sterilized mortar, and the powder thus obtained is introduced in small amounts into the nose of an animal. The results of these experiments, which are indicated in the subjoined table, show that the virus of pest supports desiccation for a long time very well when it is in an albuminous medium, as the pulp of the spleen or any other organ. Under such conditions it attenuates very slowly; in fact, in the first two or three weeks scarcely at all. On the other hand, if it is not protected, as in the case of dried cultures in infusorial earth, its virulence diminishes rapidly, so that in three weeks, inoculated into the nose of animals, the dried cultures produced no morbid effect.

Plague-pneumonia produced by nasal inoculations of dried virus.

Splenic pulp.		Dried cultures in infusorial earth.	
Duration of desiccation.	Duration of sickness.	Duration of desiccation.	Duration of sickness.
	Days.		Days.
5 days.....	4½	2 days.....	7
8 days.....	4	5 days.....	6½
12 days.....	4½	7 days.....	(b)
14 days.....	5	19 days.....	12
20 days.....	4½	31 days.....	(b)
26 days.....	4½		
30 days.....	6½		
33 days.....	6½		
38 days.....	6½		
44 days.....	(b)		

a La Pneumonie pesteuse experimentale. Annales de l'Institut Pasteur, May, 1899, page 392.
b Resisted.

HANKIN (a) states that as far as his researches go they tend to show that the bubonic microbe, whether derived from cultures or the organs of deceased animals, and whether placed in cotton, or sheep's wool, or gunny cloth, uniformly die out in six days. He states, however, that owing to the fact that epidemiological evidence tends to show that clothing may in rare cases convey the infection he is unwilling to draw any definite conclusions from his researches at present.

THE GERMAN PLAGUE COMMISSION (b) (Gaffky, Sticker, Pfeiffer, Dieudonné) used the organism from agar-agar, and bouillon cultures; also sputum from plague-pneumonia, peritoneal exudate from guinea pigs, etc. This infectious material was placed on various objects, as glass, silk threads, filter paper, fabrics, earth, etc., and permitted to dry. The plague-infected objects were placed in Petri dishes and put in a dark corner of the laboratory to dry out. As a rule, the infected pieces were turned over from time to time to hasten the drying.

The temperature of the laboratory during these experiments was about 29° to 31° C. The objects were well protected from the light. Some were kept in an improvised ice box, in which the temperature was about 22° C. and the air saturated with moisture. The proof that the organism was still alive could, unfortunately, only be made by inoculation of mice and not by means of cultures. Small pieces of the infected objects were taken from time to time and placed under the skin of a mouse. The following are the results:

Infected with pure culture.

Object.	Temperature.	Longest time in days the organism lived.
Glass	30° to 32° C.	3
Glass in desiccator.....	do	1
Filter paper.....	do	3
Filter paper in desiccator.....	do	1
Silk thread.....	do	5
Silk thread in desiccator.....	do	1
Piece of silk.....	do	6
Piece of silk in desiccator.....	do	1
Piece of wool.....	do	6
Piece of wool in desiccator.....	do	2
Large piece of linen.....	do	7

Infected with plague organs.

Glass.....	{ 30° to 32° C.	2
	{ 22° to 24° C.	2
Filter paper.....	{ 30° to 32° C.	2
	{ 22° to 24° C.	2
Silk thread.....	{ 30° to 32° C.	2
	{ 22° to 24° C.	2
Piece of wool	{ 30° to 32° C.	6
	{ 22° to 24° C.	6
Large piece of gauze.....	{ 30° to 32° C.	6
	{ 22° to 24° C.	6
Large piece of linen.....	{ 30° to 32° C.	6
	{ 22° to 24° C.	8

a The plague in India, 1896, 1897, Vol. II, appendices I to T, page 10, *et seq.*

b Deutsche Pestkommission. Kaiserliche Gesundheitsamt, Bd. 16. Berlin, 1899, page 274, *et seq.*

Sputum from plague-pneumonia.

Object.	Temperature.	Longest time in days the organism lived.
Glass.....	30° to 32° C.	1
Silk thread.....	do.....	3
Piece of wool.....	do.....	6

Pus from bubo.

Glass.....	30° to 32° C.	6
Silk thread.....	do.....	1
Piece of wool.....	do.....	1

Peritoneal exudate from plague-infected guinea pig.

Silk thread.....	29° to 31° C.	
Filter paper.....	do.....	
Piece of silk.....	do.....	(a)
Piece of wool.....	do.....	
Glass tube.....	do.....	(b)

a Still virulent on the second day.*b* Dead on the seventh day.

The longest observation made by the commission on the viability of the plague bacillus was therefore eight days.

It made no difference whether cultures one to two days' old, or eight-day-old cultures were used, as far as the resistance to drying was concerned.

Pieces of the skin of mice dead of plague were also kept and it was found that they had lost their infecting power after four and six days. In dry organs the pest bacillus soon died. The longest time observed was in pieces of liver kept in sealed glass tubes. Here they lived seven days; after seven days no result was obtained.

Sterilized feces were inoculated with a bouillon culture of plague obtained fresh from a corpse and then saturated with silk threads, wool, and silk fabrics. After drying they were wrapped in sterile filter paper and, inclosed in cotton, were packed in a box and kept in the laboratory at about 29° C. After four days the pest bacillus was alive on all the test objects; after six days only in the wool, and after eight days dead in all.

The energetic drying in the desiccator over sulphuric acid hastened the death of the organism.

On account of the great importance of this question, several more tests were made, at lower temperatures, by the committee after its return to Germany. It was there possible, on account of the more favorable laboratory conditions, to make cultures as well as inoculations into mice in order to establish the life and death of the bacillus. The bacillus was kept alive in a room at 15° to 18° C. for twenty-eight days. After thirty-three days no more growth was obtained. Even after twenty-four days the growth was very sparse. The pathogenicity for mice died out much quicker after eighteen days.

“Apparently then the bacillus pestis is very sensitive to rapid and energetic drying as occurs by higher temperatures and in the desiccator.

“Slower drying at lower temperatures is much less deadly. In the climate of Germany, therefore, a rapid death of the organism in fabrics, etc., is not to be expected, as occurs in the tropics. In the hot countries no live bacilli were found in the dust.”

EDUARDO GERMANO (*a*) made many observations to determine this question. He worked with a culture from the Breslauer Hygienic Institute obtained through Kral, and which had been grown through many generations in vitro. He used bouillon suspensions of agar cultures and exposed the infected objects in Petri dishes at room temperature, 16° to 20° C.

Room dust mixed with an emulsion of plague bacilli.

Time.	Moist.	Dry.	Dried over sulphuric acid.
1 day.....	—	—	—

Fine sand mixed with emulsion of plague bacilli.

Time.	Moist.	Dry.	Dried over sulphuric acid.	Time.	Moist.	Dry.	Dried over sulphuric acid.
1 day.....	+	(a)+	—	20 days.....	+	—	—
5 days.....	+	—	—	25 days.....	+	—	—
5 days.....	+	—	—	30 days.....	+	—	—
8 days.....	+	—	—	40 days.....	+	—	—
12 days.....	+	—	—	50 days.....	+	—	—
16 days.....	+	—	—	60 days.....	(b)+	—	—

a The dust is not yet dry.

b The number of colonies is diminished at least to the $\frac{1}{10}$ part of the original.

Loam (Humus boden) mixed with emulsion of plague bacillus.

Time.	Moist.	Dry.	Over H ₂ SO ₄ .	Remarks.
1 day.....	+	—	—	
3 days.....	+			
5 days.....	+			
8 days.....	+			
12 days.....	+			
16 days.....	+			
20 days.....	+			
25 days.....	+			
30 days.....	+			
40 days.....	+			
50 days.....	+			
60 days.....	+			

a Die Uebertragung von Infektionskrankheiten durch die Luft. Dr. Eduardo Germano. Zeitschrift für Hygiene, Vol. XXVI, 1897; 281.

Tufa (Tuff boden) rubbed up with plague bacillus.

Time.	Moist.	Dry.	Over H ₂ SO ₄	Remarks.
1 day.....	+	—	—	
5 days.....	+			
8 days.....	+			
12 days.....	+			
16 days.....	+			
20 days.....	+			
25 days.....	+			
30 days.....	+			
40 days.....	+			
50 days.....	+			
60 days.....	+			

Marly loam (Löss) rubbed up with plague bacillus.

Time.	Moist.	Dry.	Over H ₂ SO ₄	Remarks.
1 day.....	+	—	—	
3 days.....	+			
5 days.....	+			
8 days.....	+			
12 days.....	+			
16 days.....	+			
20 days.....	+			
25 days.....	+			
30 days.....	+			
40 days.....	+			
50 days.....	+			
60 days.....	+			

Brick dust mixed up with plague bacillus.

Time.	Moist.	Dry.	Over H ₂ SO ₄	Remarks.
1 day.....	+	+	—	
3 days.....	+	—		
5 days.....	+			
8 days.....	+			
12 days.....	+			
16 days.....	+			
20 days.....	+			
25 days.....	+			
30 days.....	+			
40 days.....	+			
50 days.....	+			
60 days.....	+			

From these tables it follows that the bacillus of plague withstands drying badly, while it remains alive a long time in moist conditions. Only in room dust did it die quickly under moist conditions. After two months, even under moist conditions, the number of bacteria was very much diminished. In addition to the test with dust, etc., a series was made with fabrics.

Square pieces of linen, wool, silk and filter paper (1 cm. square) were sterilized and saturated with a bouillon emulsion of culture. They were placed in Petri dishes and were exposed in the desiccator and in the room. Those that were to remain moist were placed in test tubes in the moist chamber.

The results were as follows :

Linen.

Time.	Moist.	Dry.	Over H ₂ SO ₄ .	Time.	Moist.	Dry.	Over H ₂ SO ₄ .
1 day.....	+	+		20 days.....	+		
3 days.....	+			25 days.....	+		
4 days.....		o		30 days.....	+		
7 days.....	+			40 days.....	+		
12 days.....	+			50 days.....	+		
16 days.....	+			60 days.....	+		

Wool.

Time.	Moist.	Dry.	Over H ₂ SO ₄	Time.	Moist.	Dry.	Over H ₂ SO ₄
1 day.....	+	+		20 days.....	+	+	+
3 days.....	+			25 days.....	+	+	+
4 days.....	+	+		30 days.....	+	+	
8 days.....	+	+		40 days.....	+		
12 days.....	+	+		50 days.....	+		
16 days.....	+	+		60 days.....	+		

Silk.

Time.	Moist.	Dry.	Over H ₂ SO ₄	Time.	Moist.	Dry.	Over H ₂ SO ₄
1 day.....	+	+	+	20 days.....	+		
3 days.....	+	+	+	25 days.....	+		
4 days.....		+	+	30 days.....	+		
8 days.....	+	—		40 days.....	+		
12 days.....	+		—	50 days.....	+		
16 days.....	+			60 days.....	+		

Filter paper.

Time.	Moist.	Dry.	Over H ₂ SO ₄ .	Time.	Moist.	Dry.	Over H ₂ SO ₄ .
1 day.....	+	+	+	20 days.....	+		
3 days.....	+		—	25 days.....	+		
4 days.....	+	+		30 days.....	+		
8 days.....	+			40 days.....	+		
12 days.....	+			50 days.....	+		
16 days.....	+			60 days.....	+		

It is seen, therefore, that the pest bacillus remains on fabrics quite a long time. In this respect it resembles the bacillus typhosus.

He draws the following conclusions : The pest bacillus has little resistance against drying. It can, therefore, not easily be carried in the dust or air. It is, therefore, not likely, if not impossible, that the disease is spread in the air. However, it may often occur that infection takes place through contact because the organism lives when not wholly dried. Therefore, infected clothing may be very dangerous.

V. DE GIAXA and B. GOSIO (*a*) made experiments in a small chamber partially ventilated and lighted, having a temperature varying from 10° to 18° C.

Strips of different fabrics, linen or wool, were infected after steam sterilization, and then suspended free or in small cabinets protected from the atmospheric dust. Some of the material was placed on large pieces of the same fabrics arranged so as to simulate a package.

The material of infection was deposited on the cloth either by rubbing with fresh agar-agar culture, in which case small particles of agar in addition to the germs adhere to the texture, or by the direct application of pus or blood from plague-infected animals.

The longest period of observation was thirty days; at the end of that time germs of plague were obtained from the pieces of cloth. In the case of pieces of cloth impregnated with pus or blood the observation was for twenty-nine days. On these the germs developed either in the open air or on cloth folded and inclosed in a box to simulate clothing packed in a trunk.

Pieces of linen thread were steeped in infected blood and exposed to desiccation in an incubator at an air circulation of 36° to 39° C.

After two, four, and five days several threads were placed in tubes of broth. Those exposed two days to desiccation developed slowly. Of those desiccated for four days, only 1 in 3 remained sterile; all those exposed for five days remained sterile.

Plague-infected blood was spread on a rabbit's skin and allowed to dry for four days. After fifteen days' exposure to a temperature varying from 12° to 16° C. (diffused light) the infected surface was scraped and the scrapings were cultivated. The plague bacillus soon developed.

As regards pathogenic action, the germ exposed to desiccation almost always showed a decided slowness in killing the animal.

EFFECT OF SUNLIGHT ON THE PLAGUE BACILLI.

ALBRECHT and BHON (*b*) exposed peritoneal exudate in a test tube (in August, in Vienna), to direct sunlight for six hours, and could not notice any marked difference in the organism.

ABEL (*c*) found that plague bacilli on cover glasses died after one hour exposure to sunlight at 30° C. (in Hamburg). Controls kept in the dark grew after six hours. Thicker spreads from agar cultures withstood sunning at 30° C., three and one-half hours.

KITASATO (*d*) found that the bacillus was killed at Hongkong in three to four hours' sunning. He used pus from buboes.

a *Ricerche sul bacillo della peste bubbonica, etc.*, *Annali d'igiene sperimentale*, 1897, No. 7, page 261.

b *Die Peste*, Müller and Poch, page 61, Vienna, 1900.

c *Zur Kenntnis des Pestbacillus*. *Centralblatt für Bakteriologie und Parasitenkunde*, etc., Bd. XXI, 1897.

d Preliminary notice of the bacillus of bubonic plague, Hongkong, 1894, July 7.

WILM (*a*) found them destroyed in four hours' exposure to the sun.

THE GERMAN PLAGUE COMMISSION (*b*) made tests in Bombay in this manner: The various objects (glass, silk threads, wool) were saturated with a virulent agar culture of the bacillus pestis. One-half of the objects were exposed to direct sunlight. The other half were laid away in a dark place for control.

In very thin layers on glass the bacillus was killed in one hour.

In thicker layers on glass they were pathogenic for mice after three hours but not after four hours.

In silk threads they were killed after three hours' sunning.

In wool they were virulent for mice after eight hours.

Other tests were made with well grown agar cultures placed in the sunlight. In one or two hours in the sun they still contained active organisms. After a whole day's sunning they were dead. The cultures are warmed very much in the sun; therefore, other tests were made by placing the agar tube in water and keeping in the sun one and one-half hours. At the end of the test the temperature of the water was 39° C. The bacillus was alive and virulent.

The following report of the experiments on the viability of the plague bacillus was published by S. L. RAPPOPORT, (*c*) St. Petersburg. The material used was allowed to soak in bouillon cultures of bacillus pestis in a dark closet for twenty-four hours, then exposed for successive days to all the sunlight obtainable, or to dry heat.

Temperature and time required to kill.

Material.	20 C. (68 F.)	36 C. (96.8 F.)	60 C. (140 F.)	80 C. (176 F.)
	<i>Days.</i>	<i>Days.</i>	<i>Minutes.</i>	<i>Minutes.</i>
Silk thread.....	19 to 24	13	75	15
Note paper.....	10 to 17	5	30	15
Filter paper.....	10 to 24	7	45	15
Linen thread.....	9 to 13	4	30	15
Woolen thread.....	13 to 23	5	60	15

VIABILITY ON VARIOUS OBJECTS.

In sputum kept fluid, the GERMAN PLAGUE COMMISSION (*d*) found the bacillus of pest infectious after ten days.

HANKIN (*e*) made researches on various articles of produce to determine their susceptibility to endanger or support the bubonic microbe. He shows that the various descriptions of flours and grain usually stored in Bombay contain no trace of the bubonic microbe and also demonstrates that grain purposely infected does not permit of the existence of the microbe for more than about four days.

a Uber die Pestepidemie in Hongkong im Jahre 1896. Hygienische Rundschau, 1897, Bd. VII.

b Loc. cit., page 277.

c Quoted by Walter Wyman, Surgeon-General Marine-Hospital Service, in "The Bubonic Plague," Treasury Department Document No. 2165.

d Kaiserliche Gesundheitsamte, Bd. 16, Berlin, 1899.

e The Plague in India, 1896, 1897, Vol. II, Appendices I-IV, page 10 *et seq.*

Hankin worked with linseed, yellow rape seed, brown rape seed, tilseed or ginjelly seed, ground nuts, castor seeds, poppy seeds, wheat (new hard red), wheat (another light variety), flour.

In order to test whether the microbe was still present in a living condition in a specimen of previously infected grain his method consisted of making an extract of this grain and injecting it into a mouse.

He used agar cultures making bouillon suspensions. About a kilogram of grain to be tested was placed in a sterile stoppered foot glass. One c. c. of the bouillon emulsion of the microbe was then poured into the foot glass, care being taken that it should fall into the center of the grain. The stopper was replaced and the foot glass was immediately violently shaken for about one-quarter of an hour.

Extracts made from the grain in this manner were inoculated into mice with the following results :

Extract of the following injected into mice.	At once after infecting the grain.	Two days after infecting the grain.	Four days after infecting the grain.	Eleven days after infecting the grain.	Thirteen days after infecting the grain.
Linseed.....	Died within 24 hours.	Died after 7 days.	Died after 15 days.	Survived.....	Survived.
Yellow rape seed.....	Died within 72 hours.	Died after 24 hours.	Survived.....	do	Died after 24 hours.
Brown rape seed	Died within 48 hours.	Died after 4 days.	do	Died after 24 hours.	Survived.
Tilseed.....	Died within 24 hours.	Survived.....	do	Died after 7 days.	Do.
Ground nuts.....	do	Died after 11 days.	do	Died after 24 hours.	Do.
Poppy seed	do	Died after 48 hours.	do	do	Do.
Wheat (new hard red).	Died within 48 hours.	Survived.....	do	do	Do.
Wheat (another kind).	Survived.....	do	do	do	Do.
Flour.....	Died within 48 hours.	Died after 48 hours.	do	do	Do.

Hankin concludes from these experiments that the bubonic microbe derived from pure cultures perishes within thirteen days after being added to the above-mentioned specimens of grain and seeds.

Further experiments were made with grain and seeds by mixing the spleen of a rat and the liver, spleen, and œdema from a mouse, both animals having died after plague infection, instead of cultures of the organism. The organs were powdered up in a mortar with powdered glass and mixed with bouillon and added to the grain and seed. The further proceedings were as above.

The result of this showed that grain infected with the organs of animals dead of the plague lost its infectious power completely within six days.

Another set of experiments was then conducted by infecting the grain with sputum from a case of pneumonic plague. Sputum was taken, in which the organism was demonstrated to exist in large numbers in very virulent condition.

These also showed the plague infection to die out from all the variety of seed and grain tested within six days.

ABEL (*a*) found living bacilli of plague in water after twenty days. He added an œse to 50 c. c. of sterile distilled and tap water.

The GERMAN PLAGUE COMMISSION (*b*) found that the organism was no longer virulent after five days in tap and ten days in distilled water.

YOKOTE (*c*) worked on the problem of how long the plague bacillus can remain alive in the dead body. He used mice for his experiments because they are very susceptible. The mice were inoculated and the presence of the bacilli demonstrated in the heart's blood of the dead animals, which were then placed in a wooden casket. Each casket was covered with a lid and then buried in a metal box filled with garden earth. From time to time water was poured on the earth so that it always had a certain moisture. The temperature of the room was taken daily. After a certain time the bodies were disinterred, the amount of moisture present in the earth surrounding the casket determined quantitatively, also examined bacteriologically and by animal experiments for pest bacilli. Mice were used for this purpose, and glycerin agar for culture medium.

On disinterring the body the amount of decomposition was noted, and by means of cover-glass preparations he determined the characters and numbers of micro-organisms present in the various organs. Plate cultures were made from the internal organs, on agar-agar, and some of the heart's blood, liver, or in case of advanced decomposition, some of the remains were inoculated into mice subcutaneously.

As a result of these experiments, he concludes that the pest bacillus loses its life and its power of infection in a relatively short time. It remains alive, at most, twenty to thirty days. This depends on the temperature. The higher the temperature, the stronger the decomposition, the shorter the life of the bacillus. In summer many saprophytes grow in the body and cause products which kill the pest bacillus. In cold winter this growth of saprophytes is less, and the pest bacillus could therefore live and maintain its virulence a longer time.

He states that his experiments are not sufficient to determine positively the length of time the pest bacillus may live in a buried body, but he gives it as his belief that the organism can not live longer in the cadaver than spore-bearing organisms because it does not have spores. It is also of interest to notice that the pest bacillus did not escape from the wooden casket into the surrounding earth. It seems, therefore, that there is no danger in the burial of pest cadavers of infecting the surrounding earth as long as the coffin is tight.

a Loc cit.

b Loc cit.

c Über die Lebensdauer der Pestbacillen in der beerdigten Tierleiche. Dr. Z. Yokote. Centralblatt für Bakteriologie und Parasitenkunde, etc., Vol. XXIII, 1898, No. 24, page 1030.

THE LENGTH OF TIME THE PEST BACILLUS WITHSTANDS DRYING.

Author.	Conditions.	Resisted.	Killed.
Kitasato.....	Dried on cover glass slips at 28° to 30° C..	1 to 36 hours....	4 days.
Wilm.	Dried on glass slips at 29° to 31° C.....	4½ days.....	After 4½ days.
	Dried in vacuo.....		3 hours.
Abel.....	Dried on various objects at 35° C.....		2 to 4 days.
	Dried on various objects in vacuo over H ₂ SO ₄ at 20° C.		2 to 4 days.
	Dried on cover slips from pus and culture at 16° to 20° C.	14 days.....	After 14 days.
	Dried on threads, in linen, in tissue at 16° to 20° C.	30 days.....	
Ficker.....	Dried on piece of fresh skin, 16° to 20° C	10 days.....	
	Dried on cover glasses in desiccator....	8 to 11 days.....	9 to 12 days.
	Dried and moistened alternately.....	20 to 36 hours..	28 to 48 hours
Batzaroff.....	Dried splenic pulp, "room temperature."	28 days.....	44 days.
	Dried cultures mixed with infusorial earth.	19 days.....	31 days.
Hankin	Dried on sheep's wool, and gunny cloth and cotton.		6 days.
German Plague Commission.	Dried on glass, filter paper, silk threads, pieces of silk and wool and linen, at 30° to 32° C. exposed both in room and in desiccator.	7 days.	
	Dried on the same objects at 30° and 32° C., and 22° to 24° C., infected with bacillus from plague organs.	8 days.	
Germano.....	Dried and kept at 15° to 18° C.....	28 days.....	33 days.
	In moist and dry room dust, 16° to 20° C.		1 day.
	Dried in fine sand and brick dust, 16° to 20° C.	1 day.....	5 days.
	Dried in loam, tufa, and marl.....		1 day.
	In moist sand, loam, tufa, marl, and brick dust at 16° to 20° C.	60 days.	
	On moist linen, wool, silk, and filter paper at 16° to 20° C.	60 days.	
	Dried on linen, 16° to 20° C.....	1 day.....	4 days.
	Dried on wool, 16° to 20° C.....	30 days.	
	Dried on silk, 16° to 20° C.....	4 days.....	8 days.
	Dried on filter paper	4 days.	
Giaxa and Gosio.....	Dried on linen and wool; agar cultures, pus and blood used. 10° to 18° C.	30 days.....	
	Dried on linen thread, 36° to 39° C.....	4 days.....	5 days.
	Rabbit skin infected with plague-infected blood, 12° to 16° C.	15 days.....	
Rosenau.....	Dried on little balls of gelatin-albumen, 20° to 23° C.	75 days.....	
	Dried on little balls of gelatin-albumen, 17° to 19° C.	75 days.....	
	Dried on crash, 17° to 19° C. and 20 to 23° C.	48 days.....	
	Dried on crash, 20° to 27° C.....	13 days.....	15 days.
	Dried on paper, 20° to 27° C.....	8 days.....	11 days.
	Dried on pine wood, 20° to 27° C.....	4 days.....	8 days.
	Dried on pine wood, 17° to 19° C.....	8 days.....	11 days.
	Dried on pine wood, 20° to 23° C.....	11 days.....	13 days.

EFFECT OF SUNLIGHT ON PLAGUE BACILLI.

Author.	Condition.	Killed.	With- stood.
			<i>Hours.</i>
Abel.....	In Hamburg, 30 C., thin spreads on cover glasses	1 hour.	
	Thicker spreads.....		3½
Kitasato	In Hongkong. Pus from buboes.....	3 to 4 hours..	
Wilm		4 hours.....	
Albrecht and Bhon	Vienna in August, peritoneal exudate.....		6
German Pest Commission..	In Bombay, thin layers on glass.....	1 hour	
	thicker layers on glass.....	4 hours	3
	silk threads.....	3 hours	
	in wool.....		8
	agar test tube culture	All day.....	2
Rappoport.....	In St. Petersburg, silk threads, 20 C.....	24 days	
	note paper, 20 C.	17 days	
	filter paper, 20 C.....	24 days	
	linen thread, 20 C.....	13 days	
	woolen thread, 20 C.	23 days	

VIABILITY OF THE ORGANISM IN VARIOUS MEDIA.

Author.	Condition.	Alive.	Dead.
		<i>Days.</i>	<i>Days.</i>
German Plague Commission..	In sputum, 30° to 32° C.....	6 to 10	
	In pieces of liver kept in sealed tube.....	7	(a)
	In sterilized feces in thread, fabrics, and paper, 29° C.	4	8
Hankin.....	On seeds, grains, and flours.....		13
Abel	In water, distilled and tap.....	20	
German Plague Commission..	In tap water.....		5
	In distilled water		10
Yokoto	In cadavers of mice, buried.....	20 to 30	

a After 7 days.

BULLETIN OF THE HYGIENIC LABORATORY,

Treasury Department, U. S. Marine-Hospital Service,

WALTER WYMAN, *Surgeon-General.*

FORMALIN DISINFECTION OF BAGGAGE

WITHOUT APPARATUS.

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WASHINGTON, D. C.

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FORMALIN DISINFECTION OF BAGGAGE WITHOUT APPARATUS.

This work was undertaken on account of the rather widespread use of formalin for the disinfection of baggage by sprinkling it on clothing in trunks, or by pouring it on absorbent material in the corners of a trunk. From what follows, it will be seen that the method is not a good one.

Formaldehyd possesses an antiseptic power much stronger than any of the organic nontoxic substances. It has, however, distinct limitations, which must be understood in order to properly apply it to practical disinfection. It is the object of this work to point out one of these limitations.

Formaldehyd (formic aldehyd) is a complex and unstable substance. It exists as a gas, in solution, and as a solid. In solution it is a powerful disinfectant. The gas formaldehyd is a valuable disinfectant, but is so unstable and so difficult to obtain in its gaseous state that its use in unskilled hands may prove unsatisfactory.

Formaldehyd exists in at least three well-recognized isomeric states :

1. Formaldehyd (formic aldehyd) is a gas at ordinary temperatures, colorless, and possessing slight odor, but having an extremely irritating effect upon the mucous membrane of the nose and conjunctiva. At a temperature of about -20° C. the gas polymerizes into paraformaldehyd, known commercially as paraform.

2. Paraform is a white substance, unctious to the touch, soluble in both water and alcohol. It consists chemically of two molecules of formaldehyd. It is this substance which is supposed to compose the commercial solutions of formaldehyd, known as formalin, formol, etc.

3. Trioxymethylen is formed by the union of three molecules of formaldehyd. It is a white powder giving off a strong odor of the gas. It is but slightly soluble in alcohol and water.

To sum up, formaldehyd exists in three states :

$\text{CH}_2\text{O}.$	$\begin{array}{c} \text{CH}_2\text{O} \\ \\ \text{CH}_2\text{O}. \end{array}$	$\begin{array}{c} \text{CH}_2\text{O} \\ \\ \text{CH}_2\text{O} \\ \\ \text{CH}_2\text{O}. \end{array}$
Formaldehyd gas.	Paraformaldehyd paraform.	Trioxymethylen.

It must be plain to all who have worked with the substance known in commerce as formalin, formol, etc., that it can not be a 40 per cent solution of the gas formaldehyd in water, as claimed. It more likely

consists of a solution of the isomeric forms of formaldehyd, especially paraformaldehyd.

Formalin solutions are unstable. In cold weather they are apt to precipitate a white substance, usually trioxymethylen. Heating and evaporating them has a tendency to change the formaldehyd to paraform.

These facts are stated to emphasize the importance of not considering commercial "formalin" as a simple solution of a gas in water. This has no bearing in using formalin as a liquid disinfectant in the same way that we would use solutions of bichloride of mercury or carbolic acid, but it has a very distinct and important bearing in gaseous disinfection with formaldehyd.

It is not the scope of the present article to dwell upon the best way to obtain the gas formaldehyd from its solution. The present work points out the difficulty of obtaining the gas without special apparatus from its solution in a closed container.

The power of formaldehyd, gas as a disinfectant depends on the chemical change which takes place between the gas and the albuminous matter of the germ. To be effective, therefore, the gas must not only be brought in direct contact with the germ, but in sufficient amount and for a sufficient length of time. In this respect it does not differ from bichloride of mercury and other chemical disinfectants. This point is especially mentioned here on account of the difficulty of obtaining formaldehyd gas in its free state, and the uncertainty as to the amount of gas evolved from its solution, important factors which must be taken into account in practical disinfection.

Formaldehyd forms a chemical compound with albuminous matter from which it is impossible to again separate it. It renders gelatin insoluble when added in small amounts. Egg albumen, to which formalin has been added, can not be coagulated by heat. This property of "fixing" organic matter makes it valuable as a re-agent in microscopy and explains some of its limitations as a disinfectant.

Methods.

Young bouillon cultures of typhoid and diphtheria, one to two days old, and older cultures of anthrax, containing spores, were used to impregnate slips of paper.

White filter paper is cut into slips about 1 inch long, by one-fourth of an inch wide and sterilized by dry heat. These are convenient to drop into test tubes. The slips are impregnated with the bouillon cultures and exposed between the blankets and sheets in the boxes.

Freshly prepared, moist slips were used throughout this work, because moist cultures are more readily killed by formaldehyd than dry ones, and it was designed throughout to make the conditions favorable for the disinfecting action of the gas to act.

The blankets and sheets used in these experiments are folded to fit the boxes and then sterilized with steam. The slips of paper contain-

ing the organisms are placed between the fold of the sterile blankets or sheets. The rule was to allow the blankets and sheets to dry. Sometimes they were used while still moist, but when this latter was done it is always specifically noted.

Three methods were adopted in placing the formalin in the boxes: 1, sprinkling; 2, pouring; 3, soaking on absorbent cotton.

Particular pains were taken in sprinkling because it was found that the result depends upon the technique of sprinkling.

The formalin was sprinkled from a pipette, drawn out so as to deliver a small drop. The solution was dropped from the pipette upon the blankets or sheets, as the case may be, spread out on the bottom of the box. The drops were evenly distributed over the surface and sufficiently far apart so as not to run together. It makes considerable difference in the results whether the formalin is poured on one place or carefully sprinkled in the manner indicated.

The time of exposure varied from twenty-four hours to five days, and is stated for each test. The boxes were ordinary pine packing cases, made reasonably gas tight by the use of impervious paper and varnish. They closely imitated the conditions presented by a trunk. In all other respects the experiments were made to imitate as closely as possible the conditions found in packing the average trunk.

After charging the boxes the covers were nailed down and left at the room temperature, which in this laboratory is about 18° to 25° C. When the time of exposure has expired the boxes are opened and the slips of paper are inoculated into bouillon without delay. The large size test tubes, two-thirds full of bouillon, were used in order to dilute the trace of formaldehyd which sometimes remains. The method of neutralizing the slips of paper after exposure to formaldehyd with ammonia before inoculating them into bouillon was not used in all cases, especially in the earlier parts of the work. From and including test No. 39 all the slips were neutralized as follows:

After the exposure the slips are held fifteen to thirty seconds in a beaker containing ammonia and then planted in bouillon. It is well to wash out the formamide formed by the combination of the formaldehyd and ammonia with a little distilled and sterile water, but that this is not necessary is shown in the following test:

Slips of filter paper impregnated with bouillon cultures of typhoid, anthrax, and diphtheria, prepared as described above, were placed in a petri dish and exposed to formaldehyd for ten minutes by placing a little pledget of cotton containing a few drops of formalin in the same dish. Some of the slips were then inoculated into bouillon with the following result:

Typhoid (3 slips), no growth.

Anthrax (3 slips), all grew.

Diphtheria (3 slips), no growth.

The others were exposed to ammonia before inoculation into bouillon, with the following result:

Typhoid (3 slips), all grew.

Anthrax (3 slips), all grew.

Diphtheria (3 slips), all grew.

It will therefore be seen that the formaldehyd inhibited but did not kill the typhoid and diphtheria, for no visible growth was obtained from the slips planted directly from their formaldehyd exposures into bouillon, whereas, those first treated with ammonia showed a good growth.

It is therefore reasonable to suppose that in some instances throughout this work the same phenomenon resulted, especially in the tests in which the trace of formaldehyd was not neutralized with ammonia. This possible error, however, does not effect the conclusions drawn from the work, because the results obtained show that formalin when used in a closed container has feeble powers of penetration and can not be recommended as a safe and effective disinfectant.

If some of the cultures exposed were inhibited and not killed this conclusion would be still more positive.

The bouillon tubes inoculated with the slips of paper are incubated at 37° C. In case a growth does not appear, they are kept at least ten days, frequently two or three weeks, before discarding.

In many instances the growth was found to be retarded. No visible growth before five to seven days.

In case the tubes showed positively the growth was always tested for purity. In few instances contamination crept in, usually a mold from the air or a rod resembling subtilis.

Control slips were always kept under equal conditions for twenty-four hours and inoculated as above to verify every test.

Viability of cultures.

The cultures used in these observations were anthrax, diphtheria, and typhoid. When exposed in bouillon cultures to heat they gave the following results:

Typhoid—

Five minutes at 65° C., growth.

Ten minutes at 65° C., growth.

Fifteen minutes at 65° C., slight growth.

Twenty minutes at 65° C., no growth.

Diphtheria—

Five minutes at 65° C., growth.

Ten minutes at 65° C., growth.

Fifteen minutes at 65° C., no growth.

Twenty minutes at 65° C., no growth.

Anthrax spores—

Two minutes in steam, growth.

Three minutes in steam, growth.

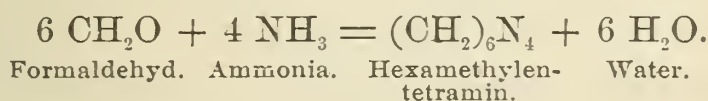
Five minutes in steam, no growth.

Eight minutes in steam, no growth.

Strength of formaldehyd used.

The same formalin was used in all these experiments. Its strength was estimated by the quantitative method given by Trillat in *La Formaldehyde et ses Applications*, 1896, and Ernst J. Lederle, Ph. D., in the *New York Medical Journal* for October 16, 1897, and is briefly as follows:

The principle on which the method is based is that ammonia and formaldehyd combine quite readily to form the compound hexamethylen-tetramin, according to the following reaction:



From the amount of ammonia required to form this compound the formaldehyd is calculated. The best results are obtained when an excess of ammonia is used, the mixture allowed to stand for at least twelve hours, with occasional shaking, and the excess of ammonia present determined by means of sulphuric acid, using coralline (rosalic acid) as an indicator.

Method of analysis.—Take the specific gravity of the solution at the room temperature.

Place 2 or 3 cubic centimeters, carefully measured, into a bottle with a glass stopper; add 50 to 60 cubic centimeters N/2 ammonia solution; shake well and let stand twelve hours, shaking occasionally. Then titrate with N/4 sulphuric acid, using coralline (rosalic acid) as an indicator.

Calculation.—The cubic centimeters of ammonia neutralized by formaldehyd = cubic centimeters of ammonia used, minus cubic centimeters of sulphuric acid.

The per cent strength of the solution =

$$\frac{2.25 \times \text{c. c. ammonia neutralized by formaldehyd.}}{\text{Grammes of solution taken.}}$$

In case the solution under examination is acid, the amount is first to be determined by a fixed alkali and a corresponding correction made in the above calculation.

In accordance with this method six estimations were made of the formalin solution used in these experiments, with the following results:

	Per cent.
Test No. 1.....	40.90
Test No. 2.....	40.02
Test No. 3.....	40.20
Test No. 4.....	40.50
Test No. 5.....	40.27
Test No. 6.....	39.60
Average	40.25

NOTE.—I desire to acknowledge the assistance in the latter part of this work rendered by Asst. Surg. L. D. Fricks, United States Marine-Hospital Service.

POURING THE FORMALIN ON ABSORBENT COTTON.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 1.

Four cubic centimeters formalin on cotton in corners of box, cultures between blankets, twenty-four hours.

One cubic centimeter formalin poured on pledgets of absorbent cotton placed in each corner of a box 15 by 15 by 15 inches. Four cubic centimeters in all. Moist freshly prepared cultures on slips of filter paper placed between blankets near the center of the box. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1.....	+	+	+
2.....	—	—	—
3.....	+	—	—
5.....	+	—	—
7.....	+	—	—
Controls.....	—	+	—

No. 2.

One hundred cubic centimeters formalin on cotton in corners of box, cultures between blankets, twenty-four hours.

Twenty-five cubic centimeters of formalin poured on pledgets of absorbent cotton in each corner of a box. One hundred cubic centimeters in all. Size of box 2 by 2 by 2 feet. Fresh moist cultures on slips of filter paper placed between layers of a blanket at various distances from the corners. The blanket was laid at the bottom of the box, smoothed out but not pressed down and no weight above it. Exposure, twenty-four hours.

Layers of blankets.	Anthrax spores.	Diphtheria.
1.....	+	+
4.....	+	+
6.....	+	+
Controls.....	+	+

No. 3.

One hundred cubic centimeters formalin on cotton in corners, cultures between layers of *sheets*, twenty-four hours.

Twenty-five cubic centimeters formalin poured on absorbent cotton tucked into each corner of a box 2 by 2 by 2 feet. One hundred cubic centimeters in all.

The freshly prepared, moist slips of filter paper, impregnated with bouillon cultures placed between layers of sheets. Box opened after twenty-four hours.

Layers of sheets.	Typhoid.	Anthrax spores.	Diphtheria.
2	+	+	+
8			
16	(c) —	(c) —	+
24			+
Controls	+	+	+

c Contaminated.

No. 4.

Twenty-five cubic centimeters formalin saturated on cotton and suspended in box. Cultures between layers of *sheets*. Twenty-four hours.

Twenty-five cubic centimeters formalin poured on a pledget of absorbent cotton, suspended in a box, 15 by 15 by 15 inches.

Fresh moist cultures on slips of filter paper placed between layers of sheets, laid without pressure at bottom of box. Twenty-four hours' exposure.

Layers of sheets.	Typhoid.	Anthrax spores.	Diphtheria.
9		+	
10			—
17		+	—
41		+	+
57		+	+
73	—	+	+
89	—	+	+
Controls	+	+	+

From the foregoing four experiments (Nos. 1, 2, 3, and 4) it is plain that even large quantities of formalin do not, in twenty-four hours, give off sufficient formaldehyd to penetrate between the layers of blankets or sheets when poured on absorbent material in the corners of, or suspended in a box. The pledgets of absorbent cotton are still moist after twenty-four hours' exposure and the formaldehyd gas has not been liberated from its solution in sufficient quantity to be effective between the layers of a blanket loosely laid on the bottom of the box. Slightly better effect is found with sheets.

The method is, therefore, not applicable for disinfection, with twenty-four hours' exposure.

POURING THE FORMALIN ON ONE SPOT—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

The following five observations, Nos. 5, 6, 7, 8, and 9, were designed to determine to what distance formalin is efficacious when poured on blankets and sheets. The formalin in these five observations was poured in a measured amount on one spot or edge of the blanket or sheet, and the cultures placed at varying distances and separated by varying layers.

In two instances, Nos. 7 and 8, the formalin was poured on a strip of absorbent cotton, laid along the edge of the blanket, instead of pouring it directly on the blanket.

No. 5.

One cubic centimeter poured on one spot, blanket, twenty-four hours.

One cubic centimeter formalin poured on one spot on a blanket at bottom of box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Slips of filter paper, moist and freshly prepared from bouillon cultures, placed between layers of blankets 2 feet away from the formalin. Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	+	+	+
2	+	+	+
3	+	(a)	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
Controls	+	+	+

a Lost.

No. 6.

One cubic centimeter poured along the edge, blanket, twenty-four hours.

One cubic centimeter formalin poured along the edge of a blanket. Moist cultures in filter paper 8 inches from formalin and between the layers, as stated in the table. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Diph- theria.
1.....	+	+
2.....	+	+
3.....	+	+
4.....	+	+
5.....	+	+
Controls.....	+	+

No. 7.

Two cubic centimeters on absorbent cotton, edge of blanket, twenty-four hours.

Two cubic centimeters formalin on a strip of absorbent cotton placed along the edge of a blanket at one end of box 15 by 15 by 15 inches. Moist cultures at a distance of 8 inches between layers of the same blanket. Box opened in twenty-four hours.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	+	+
2.....	+	+
3.....	+	+
4.....	+	+
Controls.....	+	+

No. 8.

Three cubic centimeters on strip of absorbent cotton, edge of blanket, twenty-four hours.

Three cubic centimeters formalin on a strip of absorbent cotton placed along the edge of a blanket at one end of box, 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches.

Fresh moist cultures placed at distances of 8 inches, 12 inches, 18 inches, and 24 inches, between layers of the same blanket.

Box opened after twenty-four hours exposure.

	Layers of blankets.	Inches—			
		8	12	18	24
Typhoid	1	+	+	+	+
Diphtheria.....	1	+	+	+	+
Typhoid	2	+	+	+	+
Diphtheria.....	2	+	+	+	+
Typhoid	3	+	+	+	+
Diphtheria.....	3	+	+	+	+
Typhoid	4	+	+	+	+
Diphtheria.....	4	+	+	+	+
Controls.....		+	+	+	+

No. 9.

Four cubic centimeters poured along the edge of sheet, twenty-four hours.

Four cubic centimeters formalin poured from a pipette on the edge of a *sheet* at bottom of box 15 by 15 by 15 inches, and fresh moist cul-

tures placed at measured distances between the several layers of the same sheet.

	Typhoid.	Anthrax Spores.	Diph- theria.
Fourth layer, 5 inches.....	—	+	—
Do. 12 inches	—	+	—
Do. 17 inches.....	+	+	—
Eighth layer, 6 inches.....	—	+	—
Do. 12 inches.....	—	+	—
Controls.....	+	+	+

It is plain from the above that formalin has no penetration through blankets when poured in one place. It seems to be equally ineffectual, whether the formalin is poured directly on the blanket, along its edge, or on absorbent material placed in the corners of, or suspended in a box, in the twenty-four hours' exposure.

A slightly better result is noticed with sheets. Typhoid and diphtheria are killed through 4 and 8 layers at a distance of 12 inches. No effect, however, was obtained with anthrax spores.

The effect of sprinkling the formalin so that the small drops may have better chance to evaporate and liberate the gas was next tried with better results.

SPRINKLING SMALL AMOUNTS OF FORMALIN ON SHEETS AND BLANKETS—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

In the following tests in which the formalin was *sprinkled*—this was carefully done from a pipette drawn out so as to deliver small drops. Pains were taken to distribute the solution in small drops, uniformly, over the surface of the blanket or sheet. The drops were far enough apart so as not to run together.

No. 10.

Twenty-five hundredths cubic centimeter formalin per square foot sprinkled on blanket, twenty-four hours exposure.

One cubic centimeter formalin sprinkled on the surface of a blanket at the bottom of a box 2 by 2 by 2 feet. The cultures on slips of filter paper, fresh and moist, placed between the layers of blankets.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	—	+	+
2	+	—	—
3	+	+	—
4	+	+	+
5	+	+	+
6	+	+	+
Controls.....	+	+	+

No. 11.

Five-tenths cubic centimeter formalin per square foot, sprinkled, blankets, twenty-four hours' exposure.

Two cubic centimeters formalin sprinkled on a blanket at the bottom of a box 2 by 2 by 2 feet. The cultures on slips of paper, moist and freshly prepared, placed between layers of blankets, exposed twenty four hours.

Layers of blankets.	Typhoid.	Anthrax spores	Diphtheria.
1.....	+	+	+
2.....	+	+	+
3.....	+	+	—
4.....	+	+	+
5.....	+	+	—
6.....	+	+	+
7.....	+	+	+
8.....	+		
9.....	+		
10.....	(a)		
11.....	+		
12.....	+		
13.....	+		
14.....	+		
15.....	+		
16.....	+		
Controls.....	+	+	+

a Lost.

No. 12.

Five-tenths cubic centimeter per square foot, sprinkled, formalin twenty-four hours.

Three cubic centimeters formalin sprinkled on blanket at bottom of a box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Fresh moist cultures on slips of filter paper. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.	Remarks.
1.....	—	+	—	<i>a</i> Small growth and retarded. Do.
2.....	+	+	—	
3.....	+	+	(a) +	
4.....	—	+	+	
5.....	+	+	—	
6.....	+	+	+	
7.....	+	+	+	
8.....	+	+	+	
9.....	+	+	+	
10.....	+	+	+	
11.....	+	+	+	
Controls..	+	+	+	

No. 13.

Six-tenths cubic centimeter per square foot, sprinkled on blanket, twenty-four hours' exposure.

One cubic centimeter formalin sprinkled on blanket at bottom of

a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper. Box opened after twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	—	+	—
2	—	+	+
3	—	+	+
4	—	+	+
5	+	+	+
Controls	+	+	+

In the foregoing four experiments, Nos. 10, 11, 12, and 13, the blankets were dry. The small amount of formalin used failed to kill diphtheria through 1 layer. In the following experiment, No. 14, the blankets were moist, the other conditions remaining the same:

No. 14.

Six-tenths cubic centimeter per square foot, *moist* blankets, twenty-four hours.

One cubic centimeter formalin sprinkled on moist blanket at bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of moist blankets. Box opened after twenty-four hours exposure. Blankets still moist.

Layers of blankets.	Typhoid.	Diph- theria.
1	+	+
2	+	—
3	+	—
4	+	+
5	+	+
Controls	+	+

In this experiment (No. 14) we have an irregular result. Small quantities of formalin produce irregular results in almost all the published works on this subject.

We may, therefore, conclude from the foregoing tests that small quantities of formalin sprinkled on blankets and sheets have practically no disinfecting power in a closed container, excepting on the spot where the solution falls, after twenty-four hours' exposure.

SPRINKLING DILUTE FORMALIN ON BLANKETS AND SHEETS—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

Small quantities of formalin having very little effect the next series of experiments were designed to determine whether small amounts of formalin diluted and well sprinkled would aid the evolution and penetration of the gas. Observations Nos. 15, 16, 17, 18, and 19 were made with 1 c. c. of formalin diluted with 4, 5, 10, 15, and 25 cubic centimeters of water, respectively.

No. 15.

Six-tenths cubic centimeters diluted per square foot, sprinkled on blankets, twenty-four hours.

One cubic centimeter of formalin diluted with 4 c. c. of distilled water sprinkled in small drops on a blanket carefully smoothed out over the bottom of a box 15 by 15 by 15 inches. Freshly prepared cultures on slips of filter paper, still moist, placed between layers of blankets. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	+	—
2	—	+	—
3	—	+	—
4	—	+	+
6	—	+	+
8	+	+	+
Controls	+	+	+

No. 16.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

One cubic centimeter diluted in 5 c. c. water; otherwise the same conditions as No. 14.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	—	+
2	—	+	+
3	—	+	+
4	—	+	—
5	—	+	—
Controls	+	+	+

No. 17.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

One cubic centimeter formalin and 15 c. c. water, sprinkled on blankets; otherwise the same conditions as Nos. 14 and 15.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	—	+
2	—	+	—
3	—	—	—
4	—	(c) +	—
.....	—	(c) +	—
Controls	+	+	+

c Contaminated.

No. 18.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

One cubic centimeter formalin and 15 c. c. water; otherwise the same conditions as Nos. 14, 15, and 16.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	—	+	—
2	(c) —	—	+
3	—	—	—
4	—	+	+
5	(d) —	—	+
Controls	+	+	+

c Contaminated.

d Lost.

No. 19.

Six-tenths cubic centimeter formalin diluted per square foot, sprinkled, blankets.

One cubic centimeter formalin, 25 c. c. water; otherwise the same conditions as Nos. 14, 15, 16, and 17.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	(c) +	—	—
2	+	+	—
3	—	+	+
4	—	+	+
5	—	+	—
Controls	+	+	+

c Contaminated.

Diluting the formalin, therefore, does not increase its efficiency when used by this method—sprinkling. Dilution appears to have somewhat the same influence that moist blankets have, viz, renders its penetration through the blankets very irregular.

Small amounts of formalin used by this method gave such unexpected results that a few more tests were made at a later date to confirm these observations and are inserted here.

No. 20.

Five-tenths cubic centimeter per square foot diluted, sprinkled, blankets.

Two cubic centimeters formalin diluted with 10 c. c. of water sprinkled on a blanket at bottom of a box 2 by 2 by 2 feet.

Fresh moist cultures on slips of filter paper placed between layers of dry blankets. Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	+	—
2	—	+	—
3	—	+	+
4	—	+	—
5	—	+	(a)
Controls	+	+	+

a Lost.

No. 21.

Five-tenths cubic centimeter per square foot, sprinkled, blankets.

Three cubic centimeters formalin diluted with 15 c. c. of distilled water sprinkled on a blanket at bottom of a box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Cultures on slips of filter paper, freshly prepared and moist, laid between layers of dry sterilized blankets.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	+	+
2	—	+	+
3	—	+	—
4	—	+	—
5	—	+	+
Controls	+	+	+

Being satisfied that small amounts of formalin used by this method are ineffectual, larger amounts were next tried.

SPRINKLING LARGER AMOUNTS OF FORMALIN ON BLANKETS AND SHEETS.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 22.

One and three-tenths per square foot, sprinkled, blankets, twenty-four hours.

Two cubic centimeters formalin sprinkled on a blanket at bottom of box 15 by 15 by 15 inches. Cultures on slips of filter paper, freshly prepared, placed between layers of blankets. Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores	Diphtheria.
1	—	—	(a) +
3	—	+	—
5	+	+	(a) +
7	+	+	+
9	+	+	+
Controls	+	+	+

(a) Retarded.

No. 23.

One and three-tenths cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Same conditions as No. 24.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	—	—
2	—	+	—
3	—	+	+
4	—	+	—
5	+	+	+
Controls	+	+	+

The 2 foregoing tests, Nos. 22 and 23, were made with dry blankets. The following one was made with moist blankets; the other conditions being similar :

No. 24.

One and three tenths cubic centimeters per square foot, sprinkled *moist* blankets, twenty-four hours.

Two cubic centimeters formalin sprinkled on a moist blanket at bottom of box 15 by 15 by 15 inches. Cultures fresh and moist as above and placed between the layers of a moist blanket. Box opened after twenty-four hours' exposure. The blankets are still moist.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	+	+	—
2	(c) +	+	—
3	—	+	+
4	—	+	+
5	—	+	+
Controls	+	+	+

c Contaminated.

It is again evident that the presence of moisture interferes with the penetration of the formaldehyd when used by this method and causes an irregular effect.

In the following test layer amounts, 2 c. c. per square foot were used :

No. 25.

Two cubic centimeters formalin per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches. Cultures fresh and moist on slips of filter

paper placed between layers of blankets. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	+	+
6.....	+	—
8.....	+	+
Controls.....	+	+

No. 26.

Two cubic centimeters formalin per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches. Cultures on slips of paper, freshly prepared and moist, between layers of blankets. Twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1.....	—	+	—
2.....	—	(c)	—
3.....	—	+	—
4.....	—	+	—
5.....	(c)	+	—
6.....	(d)	+	+
Controls.....	+	+	+

c Contaminated. d Lost.

No. 27.

Two cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches. Fresh bouillon cultures of the organism placed on slips of filter paper, twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	—	—
Controls.....	+	+

It is seen from Nos. 25, 26, and 27, that 2 c. c. of formalin per square foot, when well sprinkled, may be depended upon to penetrate through four layers of blankets and kill typhoid and diphtheria cultures, but it

has no effect at all upon anthrax spores, though separated by only a single layer.

The next tests were made to determine whether diluting the larger amounts of formalin before sprinkling it increased its power of penetration.

LARGER AMOUNTS OF FORMALIN DILUTED AND SPRINKLED.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 28.

Two cubic centimeters diluted per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin diluted with 5 c. c. of water sprinkled on a blanket on the bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of blankets. Box opened after twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	—	—
Controls.....	+	+

No. 29.

Two cubic centimeters formalin diluted per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters formalin diluted with 10 c. c. of water; other conditions the same as No. 28.

Layers of blankets.	Typhoid.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	—	—
Controls.....	+	+

No. 30.

Two cubic centimeters diluted per square foot, sprinkled, blankets, twenty-four hours.

Three cubic centimeters diluted with 15 c. c. of water; other conditions same as Nos. 28 and 29.

Layers of blankets.	Anthrax spores.	Diphtheria.
1.....	—	—
2.....	—	—
3.....	—	—
4.....	—	—
5.....	+	—
Controls.....	+	+

From Nos. 28, 29, and 30, it appears that 2 c. c. of formalin diluted with 5, 10, and 15 c. c. of water will kill nonspore-bearing organisms, such as typhoid and diphtheria, through 4 layers of blanket, the same as when undiluted.

STILL LARGER AMOUNTS OF FORMALIN, SPRINKLED.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 31.

Two and seven-tenths cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Four cubic centimeters formalin sprinkled on blanket laid in the bottom of a box 15 by 15 by 15 inches. Slips of filter paper impregnated with the cultures, placed while fresh and moist between the blankets. Box opened after twenty-four hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2.....	—	+	—
4.....	—	+	—
6.....	—	+	+
8.....	—	+	+
10.....	—	+	+
12.....	—	+	+
Controls.....	+	+	+

No. 32.

Two and seven-tenths cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Four cubic centimeters formalin very carefully sprinkled upon blanket at bottom of a box 15 by 15 by 15 inches. Slips of filter paper

impregnated with the cultures placed while still moist between the layers of blankets.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	—	—
6	+	+	—
8	+	+	—
10	+	+	—
Controls	+	+	+

No. 33.

Two and five-tenths cubic centimeters per square foot, sprinkled on blankets, twenty-six hours.

Ten cubic centimeters formalin sprinkled on blanket at bottom of a box 2 by 2 by 2 feet. Cultures on slips of filter paper, moist and freshly prepared from bouillon. Box opened after twenty-six hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
1	—	—	
2	+	(c)	
3	—	—	—
4	—	+	—
5	—	+	—
6	—	+	—
7			—
8			+
Controls	+	+	+

The effects of 2 and 2.7 c. c. formalin per square foot being quite constant through 4 layers of blankets, and often penetrating through 5 and 8 layers for nonspore-bearing forms of organisms, another trial was now made to contrast the effect of using the same amount of formalin and keeping all the other conditions constant, except pouring the formalin on the blanket instead of sprinkling.

Special attention is called to experiment No. 33. In this test a growth was obtained from typhoid through 2 layers of blanket. Such irregularities occasionally creeping in make an element of uncertainty that renders this method of disinfection very questionable. A good method of disinfection must have no exceptions. It must invariably kill.

ANOTHER TEST BY POURING INSTEAD OF SPRINKLING.—TIME OF EXPOSURE, TWENTY-FOUR HOURS.

No. 34.

Two and seven-tenths cubic centimeters per square foot, blankets, poured, not sprinkled, twenty-four hours.

Four cubic centimeters formalin, poured, not sprinkled, on a blanket laid on the bottom of a box 15 by 15 by 15 inches.

Diphtheria and typhoid cultures from 36-hours-old bouillon growths. Anthrax from six days, containing spores.

Slips of filter paper impregnated with cultures and placed fresh and moist between the layers of sterilized blankets. Box opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	+	—
4	+	+	—
6	—	+	—
8	+	+	+
Controls	+	+	+

This experiment again emphasizes the importance of sprinkling the formalin in small drops; otherwise the effect is irregular and uncertain.

CULTURES IN COAT POCKET.

No. 35.

Two and seven-tenths per square foot, cultures in coat pocket, twenty-four hours.

Four cubic centimeters formalin sprinkled on a blanket laid on bottom of a box 15 by 15 by 15 inches. The cultures wrapped in sterile paper and placed in the pocket of a coat which was folded and packed in the box so that the cultures were in the inside.

Nature of experiment.	Typhoid.	Anthrax spores.	Diphtheria.
Cultures in coat pocket.....	—	+	—
Do.....	—	+	—
Do.....	—	+	—
Controls.....	+	+	+

Experiment No. 35 gives expected result with nonsporulating organism, but had no effect on anthrax.

FORMALIN SPRINKLED FOR DOWNWARD EFFECT.—TIME OF EXPOSURE,
TWENTY-FOUR HOURS.

No. 36.

Two and seven-tenths cubic centimeters per square foot, blankets, downward effect. twenty-four hours.

All the foregoing observations having been made by sprinkling the formalin on blankets or sheets at the bottom of the box, and the cultures placed between other blankets or sheets laid over this, this observation was made with these conditions reversed—that is, the cultures were placed between the layers of blanket at the bottom of the box and 4 c. c. formalin sprinkled above. About the same effect was obtained.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
2	—	—	—
4	+	+	—
6	+	—	—
8	+	+	+
10	+	+	+
Controls	+	—	+

SPRINKLING LARE AMOUNTS.—TWENTY-FOUR HOURS' EXPOSURE.

No. 37.

Four cubic centimeters per square foot, sprinkled, blankets, twenty-four hours.

Six cubic centimeters formalin sprinkled on blanket at bottom of box 15 by 15 by 15 inches. Cultures fresh and moist placed between layers of blankets as in the other tests.

Layers of blankets.	Typhoid.	Diph- theria.
2	—	—
4	—	—
6	+	—
8	+	—
10	+	—
Controls	+	—

No. 38.

Four and two-tenths cubic centimeters per square foot, sprinkled on blankets, twenty-four hours.

Twenty-five cubic centimeters formalin sprinkled on a blanket in bottom of a box 3 feet 4 inches by 1 foot 9 inches by 1 foot 9 inches. Cul-

tures on slips of filter paper, moist and freshly prepared. Opened after twenty-four hours.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
1	—	—	—
2	—	—	(a)
3	—	—	—
4	—	—	—
5	—	+	—
6	—	+	+
7	—	(a)	—
8	—	+	—
9	—	+	—
10	—	+	—
Controls	+	+	+

a Lost.

It appears that increasing the amount of formalin does not correspondingly increase the power of penetration. Four cubic centimeters per square foot is not more effective than 2.5 c. c. per square foot in twenty-four hours' exposure, although more constant in its results.

The next series of experiments were made to determine whether increasing the time would increase the efficiency by this method.

From test No. 39 all the slips were neutralized with ammonia after exposure and before planting in bouillon.

INCREASING THE TIME OF EXPOSURE FROM TWENTY-FOUR HOURS TO FIVE DAYS, ETC.—FORMALIN ON ABSORBENT COTTON IN CORNERS OF BOX.

No. 39.

Ten cubic centimeters formalin in corners of box. Cultures between layers of blanket, five days.

Two and one-half cubic centimeters formalin poured on pledgets of absorbent cotton in each corner of a box 15 by 15 by 15 inches. Ten cubic centimeters in all. Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of a dry blanket laid on the bottom of the box without pressure.

On opening, after an exposure of five days, the cotton was found to be dry and contained a deposit of paraform.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
2	+	+	+
4	+	+	+
6	+	+	+
8	+	+	+
Controls	+	+	+

No. 40.

Fifteen cubic centimeters formalin in corners of box. Cultures between layers of blankets, five days.

Three and one-half cubic centimeters formalin poured on pledgets of absorbent cotton in each corner of a box 15 by 15 by 15 inches. Fifteen cubic centimeters in all. Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of a dry blanket laid on the bottom of the box without pressure.

On opening, after an exposure of five days, the cotton was found to be dry and contained a deposit of paraform.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	+	+	—
4	+	+	—
6	+	+	+
8	+	+	+
Controls	+	+	+

No. 41.

Twenty-five cubic centimeters formalin in corners of box. Cultures between layers of blanket, five days.

Six and one-fourth cubic centimeters formalin poured on pledgets of absorbent cotton in each corner of a box 15 by 15 by 15 inches. Twenty-five cubic centimeters in all. Slips of filter paper moist and freshly prepared from bouillon culture were placed between layers of a dry blanket laid on the bottom of the box without pressure.

On opening, after an exposure of five days, the cotton was found to be dry and contained a deposit of paraform.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	+	+	+
4	+	+	+
6	+	+	+
8	+	+	+
Controls	+	+	+

From Nos. 39, 40, and 41, it is plain that even five days' exposure, with the formalin in cotton in the corners of the box has no effect. The formaldehyd was deposited as paraform and not enough of the gas was liberated to destroy typhoid and diphtheria. This method is therefore totally inapplicable.

Further trials were then conducted by the method of sprinkling, but increasing the time of exposure to two, three, four, and five days.

FORMALIN SPRINKLED ON BLANKET.—TIME OF EXPOSURE, TWO TO FIVE DAYS.

No. 42.

One and three-tenths cubic centimeters formalin per square foot. Cultures between layers of blankets, seventy-two hours.

Two cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches. Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of dry blankets, seventy-two hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	(a) +	—
4	—	+	+
6	—	+	+
8	+	+	+
Controls	+	+	+

(a) Light growth and retarded.

No. 43.

One and three-tenths cubic centimeters formalin per square foot. Cultures between layers of blankets, ninety hours.

Two cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	+	—
4	—	+	+
6	—	+	+
8	—	+	+
Controls	+	+	+

Experiments Nos. 42 and 43 plainly show that small amounts of formalin sprinkled on blankets and exposed seventy-two and ninety hours do not kill anthrax spores through 2 layers of blanket nor diphtheria through 4 layers.

Larger amounts of formalin were next used.

No. 44.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blanket, forty-eight hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, forty-eight hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	(a) +	—
6	—	+	—
8	—	+	—
Controls	+	+	+

a Retarded five days—very light growth.

No. 45.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blanket, seventy-two hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, seventy-two hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	+	—
6	—	+	—
8	+	+	—
Controls	+	+	+

No. 46.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blankets, ninety hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	—	—
4	—	+	—
6	—	+	+
8	—	+	+
Controls	+	+	+

No. 47.

Two and seven-tenths cubic centimeters formalin per square foot. Cultures between layers of blanket, ninety hours.

Four cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper, moist and freshly prepared from bouillon cultures, were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diphtheria.
2	—	(a)+	—
4	—	+	—
6	—	+	—
8	—	+	+
Controls	+	—	+

(a) Light growth.

From the foregoing four tests, Nos. 44 to 47, it will be seen that 2.7 c. c. of formalin per square foot will not always penetrate through 2 layers of blankets and never through more than 4 for anthrax spores, and for nonspore-bearing organisms it can not be depended upon through more than 3 layers.

It is therefore concluded that increasing the time of exposure does not increase the power of penetration through blankets.

However, to make the work complete still larger amounts of formalin were used as follows :

LARGE AMOUNTS OF FORMALIN SPRINKLED ON BLANKETS.—TIME OF EXPOSURE, NINETY HOURS.

No. 48.

Four cubic centimeters formalin per square foot. Cultures between layers of blankets, ninety hours.

Six cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches. Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores	Diphtheria.
2	—	—	—
4	—	—	—
6	—	+	—
8	—	+	+
Controls	+	+	+

No. 49.

Four cubic centimeters formalin per square foot. Cultures between layers of blanket, ninety hours.

Six cubic centimeters formalin sprinkled on a blanket at the bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
2	—	—	—
4	—	—	—
6	—	+	+
8	+	+	+
10	+	+	—
Controls	—	—	—

No. 50.

Five cubic centimeters formalin per square foot. Cultures between layers of blanket, ninety hours.

Eight cubic centimeters formalin sprinkled on a blanket at bottom of a box 15 by 15 by 15 inches.

Slips of filter paper moist and freshly prepared from bouillon cultures were placed between layers of dry blankets, ninety hours' exposure.

Layers of blankets.	Typhoid.	Anthrax spores.	Diph- theria.
2	—	—	—
6	—	(a) —	—
8	—	+	—
10	+	+	—
12	+	+	+
Controls	—	+	+

a Small growth.

From Nos. 48, 49 and 50 more constant results are obtained with 4 and 5 c. c. formalin per square foot with ninety hours' exposure than with 2.5 c. c. per square foot.

Cultures were always killed through 4 layers of blankets but not through 6.

It is therefore plain that increasing the time of exposure with this method over twenty-four hours does not correspondingly increase its efficiency.

A few further experiments were made to test its penetration through cotton fabrics with the following results:

SPRINKLED ON SHEETS, MUSLIN, AND CHEESE CLOTH.—TIME OF EXPOSURE, TWO TO THREE DAYS.

No. 51.

Two and seven-tenths cubic centimeters formalin per square foot, sprinkled, sheets, forty-eight hours.

Four cubic centimeters formalin sprinkled on sheet at bottom of a box 15 by 15 by 15 inches. Cultures fresh and moist on slips of filter paper placed between layers of sheet. Box opened after forty-eight hours, culture held in ammonia gas one-half minute and then planted in bouillon.

Layers of sheets.	Typhoid.	Anthrax.	Diphtheria.
2	—	—	—
4	—	—	—
18	—	+	—
20	—	+	—
22	—	+	+
24	—	+	+
26	—	+	+
28	—	+	+
Controls	+	+	+

From No. 51 it is seen that formalin will penetrate through about 15 layers of sheets, which, however, does not equal in bulk 3 layers of blankets.

No. 52.

Two and seven-tenths cubic centimeters formalin per square foot, sprinkled, cheese cloth, seventy-two hours.

Four cubic centimeters formalin sprinkled on cheese cloth at bottom of a box 15 by 15 by 15 inches. Cultures freshly prepared and moist on slips of filter paper placed between the layers of the cheese cloth. Box opened after seventy-two hours, cultures held in ammonia gas one-half minute and then planted in bouillon.

Layers of cloth.	Typhoid.	Diphtheria.
26.....	—	+
38.....	+	+
51.....	—	+
64.....	+	+
78.....	+	+
88.....	+	+
Controls	+	+

No. 53.

One and twenty-five hundredths cubic centimeters per square foot, sprinkled, moist *sheets*, forty-eight hours.

Five cubic centimeters formalin sprinkled on sheet at bottom of a box 2 by 2 by 2 feet. Cultures on slips of filter paper, moist. Sheets still moist from sterilizer, forty-eight hours' exposure.

Layers of sheets.	Typhoid.	Anthrax spores.	Diphtheria.
9	—	—	—
10	—	—	—
11	—	—	—
12	—	—	—
14	—	—	—
15	—	—	—
16	—	—	—
17	—	—	—
18	—	—	—
19	—	—	—
Controls	+	+	+

No. 54.

Four cubic centimeters per square foot, sprinkled, cheese cloth, seventy-two hours.

Six cubic centimeters formalin sprinkled on a sheet at bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of sheet, three days' exposure.

Layers.	Typhoid.	Diphtheria.	Subtilis spores.
20	—	—	—
40	—	—	—
60	—	—	—
80	—	—	—
Controls	+	—	+

No. 55.

Two and seven-tenths cubic centimeters per square foot, sprinkled, sheets, seventy-two hours.

Four cubic centimeters formalin sprinkled on a sheet on bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of sheet, three days' exposure.

Layers.	Typhoid.	Diphtheria.	Subtilis spores.
8	—	—	+
15	—	—	+
Controls	+	+	+

Two and seven-tenths centimeters per square foot, sprinkled, muslin, seventy-two hours.

Four cubic centimeters formalin sprinkled on sheet at bottom of a box 15 by 15 by 15 inches. Fresh moist cultures on slips of filter paper placed between layers of muslin, three days' exposure.

Layers.	Typhoid.	Diph- theria.	Subtilis spores.
10	+	—	+
20	+	—	+
30	+	—	+
40	+	—	+
50	+	+	+
Controls	+	+	+

CONCLUSIONS.

Large quantities of formalin poured on absorbent material in the corners of or suspended in a box have practically no disinfecting power between layers of blankets or sheets, even after five days' exposure.

The formalin neither evaporates nor liberates its formaldehyd in a closed container at ordinary temperatures (18 to 25° C.) in twenty-four hours.

In two to five days the fluid will evaporate, but the formaldehyd is deposited as paraform on the spot where it was poured.

This method of pouring the formalin on absorbent material is therefore totally inapplicable to the disinfection of baggage, in a closed container.

Sprinkling formalin on baggage in a trunk or box may be depended upon to kill nonspore-bearing organisms, such as typhoid and diphtheria, through 3 layers of blankets.

The method, however, is not a reliable one unless a sufficient quantity of formalin is sprinkled in small drops uniformly over the surface and between many layers.

It can not be depended upon to kill anthrax spores through 1 layer of blanket.

Formaldehyd is given off from its aqueous solution in very small amounts at ordinary temperatures.

The evaporation of the solution in a closed container is slow and uncertain.

In using this method, therefore, great care must be exercised to sprinkle the formalin uniformly, and in small drops. It is best to keep the container in a warm place to facilitate the evolution of the gas.

Use not less than 4 c. c. of formalin, containing 40 per cent formaldehyd, over every square foot of surface between every third layer of

blankets or their equivalent. Somewhat less will suffice for linen and cotton goods.

This means the use of from 50 to 100 c. c. per cubic foot.

Increasing the amount from 2.5 c. c. to 4 c. c. per square foot does not correspondingly increase its power of penetration, but gives constant results.

Increasing the time of exposure over twenty-four hours does not increase the penetrating power of the disinfectant.

Pouring the liquid in one place, and splashing the one liquid on with brushes will not suffice. The formalin must be carefully sprinkled in small drops and distributed uniformly over the surface, between many layers.

Excessive moisture of the fabrics interferes with penetration and gives irregular results.

Formalin, as sold commercially, has an acid reaction. This is sometimes excessive and ruins colors and fabrics. Pure formalin spots and "fixes" leather, and injures certain dyes. Even pure water will spot some delicate silks and colors. Care must therefore be exercised as to the articles with which the formalin comes in contact.

As far as known, all our quarantinable diseases are caused by non-spore-bearing organisms, and our object is to disinfect, not sterilize. However, the quarantine regulations of the United States require the use of chemical and physical agents sufficient to destroy spores. This excess is justified as an element of safety to secure good results in practical work on a large scale. This method of disinfection by sprinkling or pouring formalin in a closed box, as here set forth, does not reach this standard, for even in large amounts it sometimes fails to kill anthrax spores through one layer of blanket, and therefore can not be considered a good method of disinfection.

BULLETIN No. 3 OF THE HYGIENIC LABORATORY,

Treasury Department, U. S. Marine-Hospital Service.

WALTER WYMAN, *Surgeon-General.*

SULPHUR DIOXIDE

AS A

GERMICIDAL AGENT.

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SULPHUR DIOXIDE AS A GERMICIDAL AGENT.

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In considering the action of sulphur dioxide or sulphurous oxide as a disinfecting or germicidal agent, it is well possibly to begin with a brief review of the nature of the substance, its physical properties, and the method of its action.

Sulphur dioxide, or sulphurous oxide, SO_2 , is the only compound of the combustion of sulphur in dry air or in oxygen gas. There are many methods of preparation, the easiest and most simple being the burning of sulphur in air. It may also be prepared by heating copper in a state of fine division as clippings or borings, with sulphuric acid, or by heating sulphur in the presence of concentrated sulphuric acid. By either of these methods a liberal and very regular evolution of the gas is obtained.

Sulphur dioxide is a colorless gas having a peculiar suffocating odor. It instantly extinguishes flame and is totally irrespirable, therefore quickly destroying animal life. It has a density of 2.11 ; a liter weighs 2.86 grams ; 100 cubic inches weigh 68.69 grains. At about 4 atmospheres, or 60 pounds pressure, it can be reduced to a liquid, and at 17.8°C . and at ordinary pressure it is reduced to a liquid condition, colorless, limpid, and very expansible by heat. Cold water dissolves more than thirty times its weight of the gas. Solutions containing sulphur dioxide remain unchanged if excluded from the action of atmospheric oxygen, but when oxygen is present the liquid is rapidly oxidized into a higher form, sulphuric hydrate, H_2SO_4 . In a dry state sulphur dioxide and oxygen gases may remain in contact for any length of time without change.

Sulphurous acid, or sulphur dioxide, has active bleaching properties. It is used in the arts for bleaching woven goods and straw. A piece of blue litmus paper plunged into moist sulphur dioxide is at first reddened and then slowly bleached. In considering its action as a disinfecting agent this bleaching property must be borne in mind.

The use of sulphur dioxide as a disinfecting agent dates back to a very remote period, far preceding that of the recognition of the bacterial origin of most contagious and infectious diseases, indeed, going

back to the time when the old "humoral" system of pathology was in full vogue; it being supposed, even in the then crude state of knowledge, that "contagion" was a living entity, the destructive effect of the gas was fully recognized. In an old book dealing with nautical matters and intended for the guidance of shipmasters it is related that after smallpox a room or apartment can be rendered safe by burning "a small fragment of brimstone" in the closed apartment. It is the impression of the writer that sulphur is one of the agents mentioned as having been tried in the cleansing of the celebrated Augean stables, prior to the employment of Hercules to complete the stupendous labor. Whether it was burned or whether it was used by other methods is not related. Therefore, sulphur as a disinfecting agent certainly has the respectability of antiquity to recommend it.

There has been much disparagement of the germicidal action of sulphur dioxide when employed for disinfecting or sanitary purposes. It is well to state in the very beginning that the agent has distinct limitations. Certainly it can not be denied that it has great activity when used intelligently and under favorable conditions. Two principal arguments have been actively exploited against the employment of sulphur dioxide, and these are, respectively, its lack of penetrating power and its destructive influence upon fabrics or textiles and tissues. But the question naturally arises, Is penetrating power a prime essential in a disinfecting agent? Take the case of a room or apartment infected by occupancy of one suffering with a contagious or infectious disease. These diseases are usually of comparatively short duration, and are not in any sense of the term lingering or long drawn out. The bedding of the patient may be soiled, but this soiling is more than apt to be superficial, and especially in the case of soiling by fecal matter. Soiling by urinary secretion may, of course, penetrate deeply, but it is fortunate from a sanitary standpoint that the elimination of active pathogenic bacteria by the kidneys is rare, and, indeed, in most diseases admits of some doubt.

A much more serious objection to the employment of sulphur dioxide is its destructive effects upon textiles and fabrics. Being employed, as has already been noted, in the arts as a bleaching agent, it is very evident that it is not adapted for the disinfection of such materials as are colored by vegetable dyes or by most of the aniline colors. The blue dyes of vegetable origin, such as the indigo derivatives, are turned red by the action of sulphur dioxide in the presence of moisture. The red dyes of vegetable origin, such as madder, are converted into a brilliant orange red; nor does the damage stop here. The strength of fabrics thus subjected to moist sulphur dioxide is seriously impaired, and this impairment of strength progresses so long as any of the agent remains in the interstices of the fabric. It is easy to understand this when it is recollected that the hydrated sulphur dioxide is converted into *sulphuric acid* by oxidation, and the destructive effects of this acid upon all fab-

rics and materials is a matter of common knowledge. Again, cotton or linen fabrics are equally injured, unless the bleaching action is arrested by some appropriate means. The piece of linen or cotton goods, especially if containing starch or size, exposed to the action of damp or moist sulphur dioxide is so acted upon that unless washed immediately after the disinfecting process it will be found to have rotted or decayed in such a manner that it can easily be shaken to pieces.

These are in the main the principal objections to the employment of sulphur dioxide as a germicidal agent and form distinctly one of its limitations. Therefore, let it be laid down as a general rule that, except in the absence of better means, sulphur dioxide should not be employed for the disinfection of fabrics or textiles, or, if so employed, the parties interested should be notified in advance that colored fabrics will probably be changed in appearance, and uncolored ones, unless washed immediately after, may be expected to become useless within a comparatively short time.

But, on the other hand, the agent has most distinct advantages. In the first place, the material for its production is accessible almost everywhere. There is hardly a crossroads store in any part of the country where a reasonable quantity of sulphur, either in the form of flowers or in rolls or sticks under the name of brimstone, can not be obtained. It is an easy matter, therefore, to close an apartment, and in a pot, pan, or other receptacle to burn a quantity of sulphur, and thus render an apartment safe from the danger of conveying infection to those who may subsequently occupy it. Again, the agent is very cheap, and it requires only a comparatively small quantity to disinfect large spaces. Notably is this the case in the holds of ships and in railway freight cars or in other vehicles which are used in the ordinary dispatch of commercial intercourse. For this purpose the agent is most effective and fills an important place in our quarantine and epidemic measures. Whether or not all contagions are air borne is a matter which is yet involved in doubt, but it would seem for practical purposes that they may be so considered. Therefore, wherever infection in its broadest sense has penetrated, conveyed by the surrounding atmosphere, there it is certain that the disinfecting agent, sulphur dioxide, being gaseous in its nature, will penetrate.

From a quarantine and epidemic standpoint it is most fortunate, too, that all the quarantinable or epidemic diseases common to man are caused by simple organisms—that is to say, those which are not of the spore-bearing variety. It will subsequently be shown that as against this class of organisms sulphur is thoroughly efficient when accompanied by moisture and when it can be brought into contact with them. The question of spores introduces another factor into the question, but, as has been said, fortunately the question of spores does not enter into quarantine measures or epidemic practices.

The quarantinable diseases, as defined by the regulations of the Treas-

ury Department of the United States, are cholera, yellow fever, typhus fever, smallpox, plague, and leprosy. To these, from a municipal or sanitary standpoint, may be added the eruptive fevers, measles, chicken pox, scarlet fever, and the dread scourge of municipalities—diphtheria. Leprosy for the present is left out of consideration, for while its causative organism is known its method of contagion or infection is as yet unsettled.

An effort has been made in the experiments that are about to be detailed to establish several points: First, the efficiency of sulphur dioxide as a disinfectant; second, the minimum percentage necessary for efficient disinfection against yellow fever, diphtheria, cholera, and plague; third, to demonstrate the minimum exposure to this minimum percentage in order that disinfection of vessels may be reduced to the smallest possible basis of expense, damage, and loss of time.

Ever since the systematic employment of sulphur dioxide as a germicidal agent it has been empirically recommended that moisture should be present and should be supplied either by the introduction of steam or water in the form of spray, or by water vaporized by the heat engendered in the combustion of the sulphur employed for the production of the disinfecting agent. This matter has been the subject of much discussion, and while generally recognized as being necessary for efficient work, there have been but few authoritative statements on the subject. Therefore, the first experiment made was with a view of solving this question. This was done after the following manner:

PRELIMINARY EXPERIMENT.

A large bell jar having an aperture for the admission of the gas was loaded with envelopes containing strips of flannel which were wet with bouillon cultures of anthrax, cholera, the colon bacillus, typhoid, diphtheria, the bacillus icteroides of Sanarelli, and the bacillus of bubonic plague. The envelopes were sealed and were then perforated with a punch to admit the access of the gas. The bell jar was mounted on a plate of ground glass and air-tight contact was obtained by the use of vaseline. Liquefied sulphur dioxide was the agent employed, and in order to insure its reaching the bell jar in a state of thorough dryness the gas was passed through two drying columns containing pumice stone wet with strong sulphuric acid. The percentage of sulphur dioxide contained in the atmosphere of the bell jar was determined at once by means of the decolorizing action of the gas upon a decinormal solution of iodine and was again determined at the conclusion of the time of exposure. The first experiment showed the presence of 9.8 per cent of dry sulphur dioxide, practically the 10 per cent demanded by the quarantine regulations of the Treasury Department. The time of exposure was forty-eight hours. At the conclusion of the experiment the quantity of gas in the atmosphere had fallen to about 9.2 per cent, showing practically that during the whole duration of the experiment

the percentage of sulphur dioxide had been between 9 and 10 per cent. The slips of flannel were then removed from their envelopes and planted into tubes of ordinary bouillon, and these tubes were then placed in the incubator at 37° C. and were noted at the end of twenty-four hours. It was then seen that all of the organisms except anthrax had been inhibited. This organism showed a normal growth, apparently not retarded in any way by the process to which it had been subjected. But observation at the end of another twenty-four hours—that is, forty-eight hours in all—showed that the effect on the other organisms had simply been inhibitory and not germicidal. There was a growth in all of the tubes of all of the organisms planted. The question may, therefore, be decided as settled that *dry* sulphur dioxide is *not* an efficient or reliable germicidal agent for quarantine or epidemic practice.

The experiments were then continued as follows:

EXPERIMENT No. 1.

Bouillon cultures of cholera, colon, diphtheria, typhoid, icteroides, and plague were exposed on slips of filter paper inclosed in sealed envelopes, which envelopes were perforated to allow access of the gas. The cubic capacity of the room used was 500 cubic feet. The percentage of sulphur dioxide, by actual determination, was 0.6 per cent. The vessel in which the sulphur was burned was immersed in another vessel containing a quantity of water, which was measured before and after the experiment, and the amount found evaporated was 150 c. c. Time of exposure, twenty-four hours.

Six cultures of each of the above organisms were placed on a shelf within the room; six each were suspended near the ceiling; two were placed on the floor of the room; one of each under a heavy carpet upon the floor.

The temperature at the beginning of the experiment was 31° C., and the highest temperature noted during the continuance of the experiment was 35° C.

The results obtained in this experiment were very uniform. No single organism survived the process. The controls in all cases grew.

EXPERIMENT No. 2.

The next experiment was identical with the preceding, the same organisms being used, except that the percentage of gas was 0.8 per cent and the time of exposure was shortened to twenty hours. The results were the same. All organisms subjected to the process were killed, while the controls all grew.

EXPERIMENT No. 3.

The next experiment was made with cultures as before, disposed upon the floor of the room, the shelf, suspended near the ceiling and under

carpet on the floor, but to obtain the effect of the gas upon spores, cultures of anthrax containing spores were exposed under the same conditions. The percentage of gas was 1.6 per cent. One hundred and fifty cubic centimeters of water were introduced into the room, after the combustion of sulphur, in the form of steam. Time, eighteen hours.

The results were that anthrax exposed under all conditions showed a growth. The other organisms were uniformly killed, while all controls showed normal growth.

EXPERIMENT No. 4.

Percentage of gas 4.25 per cent; time, sixteen hours. Cultures, anthrax, cholera, colon bacillus, diphtheria, typhoid, icteroides, and plague, were exposed in envelopes upon the shelf, and floor of the room, under carpet, and enveloped under four layers of blanket. Of those exposed on the shelf all were killed. Of those exposed under the carpet anthrax grew while the others were uniformly killed. Of those enveloped in the blanket anthrax, typhoid and icteroides grew; cholera, colon, diphtheria, and plague, were killed. Controls to this experiment grew uniformly.

In the experiments involving the use of a small percentage of gas spore-bearing organisms, as anthrax, were not employed, the design being to observe the effect of low percentages of the gas upon organisms interesting from a quarantine or epidemic point of view. The results would seem to indicate that provided a proper quantity of moisture be present, even these low percentages are efficient against ordinary pathogenic organisms.

Numerous experiments have been made with various percentages of the gas to fulfill the third indication laid down for these experiments, namely, to demonstrate the minimum exposure to minimum percentage.

The results of these experiments have been uniformly to show that, while low percentages of the gas have been uniformly efficient as a disinfecting agent, it has been found impossible to produce germicidal effects within any very short period. The times tried have been, respectively; two hours, four hours, six hours, eight hours, twelve hours, fourteen hours, and sixteen hours. Sixteen hours, as has been shown above, have given good results with comparatively low percentages of gas, but uniform germicidal effects have not been obtained under this time. This would seem to indicate that a certain time is necessary to allow the gas to penetrate and remain in contact with the organisms which it is designed to destroy, but, above all, the experiments have demonstrated that it is possible to employ the gas as a germicidal agent in much lower percentages and for considerably shorter term of exposure than is now required by the regulations of the Treasury Department of the United States. Four and one-quarter per cent has been the maximum quantity of gas used; twenty-four hours the longest time. While the time may have been shortened materially by the employment of this

percentage, it is still believed that twenty-four hours should be the minimum time.

Now let us see what practical bearing these results may have upon quarantine practice. The regulations require for the holds of vessels, either iron or wood, 10 per cent volume of gas, and forty-eight to seventy-two hours' exposure in the case of wooden vessels; twenty-four to forty-eight hours in the case of iron vessels. This percentage of gas can only be obtained by the use of special apparatus in the nature of a specially constructed furnace and a fan or blower driven by an engine to aspirate the gas from the furnace as fast as formed and introduce it into the hold of a ship undergoing disinfection. Investigation of many quarantine stations has demonstrated that in none of them was 10 per cent sulphur dioxide used. In many of them the percentage ranged from 2.50 to 6 per cent. So far as the record shows there is no instance of a ship once having undergone disinfection at a national or State quarantine station having imparted the infection of the disease which was principally the subject of quarantine, yellow fever, to any person or to any community subsequently in contact therewith.

It would seem that as a result of these experiments there might be an essential change made in the quarantine regulations upon this point. Five per cent sulphur dioxide intelligently applied for twenty four hours would seem to be all that would be necessary. This percentage of gas, or very near it, can be obtained by the old method of the combustion of sulphur in pots which can be placed in the holds or other apartments of ships which it is desired to disinfect. And the time can be shortened from seventy-two hours as a maximum to probably twenty-four hours as a maximum instead of a minimum, in all cases.

The above experiments having served to show the minimum percentage of gas necessary to effect certain disinfecting results and the minimum time necessary to arrive at these same facts, it would now be well to consider the means for the practical application of sulphur dioxide as a disinfecting agent.

Several methods may be employed. Let us commence with the presumption that we desire to obtain a 5 per cent atmosphere in a given space. How can this be arrived at? As has been mentioned heretofore, there are numerous methods, the burning of sulphur, the production of sulphur dioxide by the action of sulphuric acid upon either copper or sulphur, and it has also been noted that under the influence of pressure or low temperatures, or the two combined, sulphur dioxide may be reduced to a liquid condition readily expansible by the application of heat. Therefore, the first method may be given as the use of liquefied sulphur dioxide in such quantities as to obtain the percentage required. It must be borne in mind here (having reference to the atomic weights of sulphur, oxygen and sulphur dioxide) that 1 pound of sulphur will produce 2 pounds of sulphur dioxide. Therefore, reversing the proposition, 2 pounds of liquefied sulphur dioxide must be used in lieu of 1 pound of

sulphur. This method is efficient and rapid, but very expensive, and, except under certain conditions, may be eliminated from our consideration of practical quarantine measures. Neither is it worth while, in this connection, to give consideration to either of the other two methods of the treatment of either sulphur or copper by sulphuric acid, as they entail the use of heat and apparatus more or less complicated. In addition to which, it is a procedure not free from danger for the uninitiated to subject concentrated sulphuric acid to the action of heat. Terrific accidents and horrible burns might easily result. But a means easy and effectual still remains to us. That is the combustion of sulphur in the presence of atmospheric oxygen.

Taking into consideration the actual weight of a given number of cubic inches of sulphur dioxide, we find that disregarding temperature and barometric pressure, and presuming that our sulphur is free from contaminations or impurities, we can obtain about 1.15 per cent of sulphur dioxide by the combustion of 1 pound of sulphur in a space of 1.000 cubic feet. Sulphur, however, is seldom pure, containing impurities in the nature of sulphate of lime and sand, and a portion of it being always oxidized to the formation of ill-defined compounds. Again, all gases have a very regular rate of expansion and a constant factor is equivalent to $\frac{1}{3000}$ for each degree centigrade from zero up. It has been noted by Surgeon Carter, M. H. S., that the holds of vessels during the process of combustion of sulphur sometime reaches 140° F., or 60° C. Presuming, therefore, on a normal temperature of 22° C. we have ranges of temperature from 22° to 60° C., or a range of 38°, giving an expansion of gas about equivalent to $\frac{4}{30}$ of the entire mass. Taking this, we have 1.03 as the obtainable percentage under normal conditions of temperature, and making allowance for the impurities noted as existing in sulphur, it might be said that in round numbers 10½ pounds of sulphur can be counted on as producing 10 per cent sulphur dioxide in 1,000 cubic feet of space. To obtain 5 per cent, therefore, is simply to use one-half or 5½ pounds. Of course, it would be prudent to allow for wastage or for imperfect combustion, and these factors one must decide for himself. It has been suggested that 20 per cent be allowed for these sources of error.

As has been seen, the presence of moisture is necessary to an effectual germicidal power on the part of sulphur dioxide. In other words, that sulphur dioxide must be converted into sulphurous acid. It is comparatively easy to arrive at the amount of water necessary for this. The atomic weight of sulphur dioxide as compared with sulphurous acid is as 64 to 82; in other words, about 20 per cent of the weight of sulphur used should be allowed for water to be volatilized or added to produce a proper hydration. Therefore, one-fifth pound, theoretically, of water should be volatilized or added for each pound of sulphur burned. Many ways suggest themselves of effecting this result. The water might be applied in the form of steam after the combustion of the sulphur. It

might be added after the combustion of the sulphur in the form of a finely divided spray, or it might be vaporized by the heat generated in the combustion of the sulphur itself. The latter method is the one that will commend itself for practical use. Of course, it might be possible to evaporate a given quantity of water and turn the resulting steam into the apartment or hold filled with sulphur dioxide, but this would require a special apparatus. The same might be said of the production of the spray, but it would require a spray of considerable power to reach to all parts which have been penetrated by the sulphur dioxide, and, as the process would have to be conducted by faith rather than by sight, it is not a commendable practice. On the contrary, the vaporization of water by the heat engendered in the burning of the sulphur is, so to speak, an automatic process.

Let the sulphur be burned in shallow pots of iron, nothing being better than the old-fashioned Dutch oven for this purpose, and let each of these containers stand in a larger vessel containing water. As the iron of the oven becomes hot, water will be vaporized and rising around the fumes of the burning sulphur, union will at once take place and nascent sulphurous acid will be the result.

Again, allowance must be made for unavoidable losses or defects. While about one-fifth would be about the theoretical requirement, let one-half pound of water be vaporized for every pound of sulphur used.

A point of much importance, which should be mentioned, is that the vessels containing the burning sulphur should not rest at the lowest point of the apartment or hold undergoing disinfection, but, as recommended by Surgeon White, the pots should be elevated in the holds of vessels on piles of ballast, which would raise them a considerable distance above the floor of the hold of the ship undergoing disinfection or the floor of the apartment which it is desired to purify. The reason for this is obvious upon consideration. Hot gases ascend and naturally fill the upper portions of the apartments; on cooling, they descend and fill the lower strata of the hold or room. Here the flame extinguishing effect of sulphur dioxide comes into play, and it is quite possible that the flames of the burning sulphur would be extinguished before the oxygen of the atmosphere was exhausted by chemical union with the sulphur. The object to be attained is very simple; to convert all of the oxygen of the contained atmosphere of the hold or apartment into sulphur dioxide by union with burning sulphur. Bearing this in mind, methods for accomplishing this end will readily suggest themselves to anyone of ordinary intelligence, certainly to anyone who has had practical experience in the matter of ship or room disinfection.

The mention of another practical point will fittingly bring this article to a close. It has come under the observation of the writer that in some places where the "pot method" of disinfection of ships was practiced, it was the habit to employ one large vessel, a caldron or wash boiler, for the combustion of the sulphur. This is wrong, for, should

the flame be extinguished by the effect of the gas, the whole process would necessarily have to be gone over. If the sulphur is divided among a considerable number of smaller containers the greater part of this danger is obviated and, there being more sources of supply, the uncertainty which always surrounds the practical diffusion of gases is neutralized.

It is therefore recommended that the combustion of sulphur should be effected in such a manner that no vessel used for the combustion should contain more than 30 to 40 pounds of the sulphur. This sulphur, too, should be in a state of fine division, and ignition should be obtained by using a liberal quantity of alcohol to start the fire in each pot.

BULLETIN No. 4 OF THE HYGIENIC LABORATORY,

Treasury Department, U. S. Marine-Hospital Service,

WALTER WYMAN, *Surgeon-General.*

VIABILITY

OF THE

BACILLUS PESTIS.

M. J. ROSENAU,

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U. S. MARINE HOSPITAL SERVICE,

WASHINGTON, D. C.

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MARINE-HOSPITAL SERVICE,

Washington, April 25, 1901.

SIR: In May of last year a preliminary note upon "The Viability of the Bacillus Pestis" was submitted and published in the "Public Health Reports," Vol. XV, No. 21.

The work has been continued without interruption in this laboratory since that time and I have now the honor to make a more complete report upon this important subject.

During my absence in Europe this work was continued by P. A. Surgeon H. D. Geddings, M. H. S., especially in relation to food stuffs, and his report is embodied in his own words.

I have also to acknowledge material help from Assistant Surgeons Currie and Long in carrying on many details of the work while they were stationed in the Laboratory.

Respectfully,

M. J. ROSENAU,

Director, Hygienic Laboratory.

The SURGEON-GENERAL,

U. S. Marine-Hospital Service,

Washington, D. C.

THE VIABILITY OF THE BACILLUS PESTIS.

[By M. J. Rosenau, Passed Assistant Surgeon, Director Hygienic Laboratory, Marine-Hospital Service.]

There are many factors that influence the life of the bacillus pestis in the outer world. Some of these influences, such as temperature, moisture, and light, are well understood. The bacillus withstands drying very badly. It can not live long in the direct, warm sunshine. High temperatures are invariably fatal.

In the light of these experiments and the published work of others we have to modify considerably our views upon the viability of the bacillus pestis. It does not always and under all circumstances die in so short a time as a few days, as the work of Kitasato and Wilm first indicated. We now know that the organism may live for months, and even years, in a test tube, on a moist albuminous medium. And the present work shows that even when dry it may live over four months, provided the temperature is cool—less than 20° C.

The bacillus of plague can in no sense be considered a tender organism, as was at first supposed. It is much easier to cultivate than the lanceolate coccus of pneumonia or the pathogenic streptococci. In this respect it resembles more closely the hardier of the hemorrhagic septicæmic group.

In this laboratory we have worked with the organism from seven sources: from Djiddah, Oporto, Rio de Janeiro, Bombay, the New York Quarantine case, Glasgow, and San Francisco. We find that all these seven varieties thrive well on artificial media under the usual laboratory conditions.

We tested the life history of this organism upon a great variety of objects and under various conditions. We attempted to imitate nature. But we can not imitate all the conditions under which the organism may exist in nature, and we ought not, therefore, to apply the experience of the laboratory too literally to the life history of the plague bacillus outside of the body. We may determine with fair certainty the length of time the bacillus may live under given conditions. But these conditions are more or less arbitrary, and to a certain extent artificial. In general terms, we can state whether it is a hardy organ-

ism, resistant to influences usually detrimental to bacterial life, or one that loses its virulence and dies quickly when removed from its natural habitat.

The bacillus of plague does not exist in nature on sterile glass cover slips, nor yet in the desiccator over concentrated sulphuric acid, which were conditions used by some authors who have reported their results on this question.

It is also necessary to call attention to certain conditions which obtained in most of these experiments, that are very far from the usual state of affairs.

The test objects were very abundantly inoculated with a pure culture of the bacillus pestis of known activity and virulence. Often the test objects were saturated. The cultures had been grown for a long time upon artificial media in the laboratory, so that their vitality was probably strongly influenced. It is a well-known fact that virulent pathogenic bacteria may at first grow very poorly upon the ordinary laboratory media, but by successive cultivation they become accustomed to the new conditions, so that they finally thrive abundantly; that is to say, they take on a sort of saprophytic existence. Such cultures would doubtless resist the various influences to which they are exposed in the laboratory tests better than another race direct from the blood or tissues. In fact, it is found that the plague bacillus in the blood and tissues from a rabbit usually dies out rather quickly when dried upon the test objects. On the contrary, bouillon cultures dried on similar objects and under similar conditions live a much longer time.

Another departure from normal conditions was the fact that all the test objects receiving the abundant inoculations of the virulent pure cultures were sterile. In other words, not only were cultures of the bacillus used that were accustomed to a saprophytic existence, but these cultures were placed upon sterile test objects and protected against contamination, so that they were relieved from that microbial symbiosis which, in the economy of nature, plays so important a part in the suppression of pathogenic micro-organisms. It is known that in organic mixtures the hardier saprophytes tend to overpower the bacillus pestis. This even occurs to a certain extent in the blood and buboes of patients sick of the disease, as well as in dead organic infusions. The bacillus pestis is not known to have synergists among the lower forms of life. But that such exist may well be inferred from analogy.

Further, different races of the plague organism have different powers of resistance to unfavorable influences. Some races of plague are hardier than others, just as some races of mankind are hardier than others. Batzeroff states, "There undoubtedly exist varieties that attenuate very quickly and die outside the living body in a relatively

short time. There are other varieties that retain their viability under similar conditions a long time." Bacteria show quite as strong a tendency to adaptability as the higher animals. They may become accustomed to influences by a gradual survival of the fittest so that they finally resist conditions that would be fatal to the parent stock.

All these facts must be borne in mind in drawing conclusions from laboratory experiments.

PLAGUE AND FOOD.

Our experiments show that food products may harbor the infective principle of plague, but according to experience food products are not much to be feared as far as their probability of carrying the infection is concerned. This latter statement does not apply to milk and its products, for milk is a good culture medium for the bacillus pestis; and we kept it alive seventeen days in cheese and seventy-two days in butter.

On the surface of food products it usually died very quickly. It did not live twenty-four hours on orange peel. We had similar results with figs and raisins and a large quantity of Chinese food products, such as smoked and dried ducks, dried oysters, dried cuttle fish, dried ducks' gizzards, ducks' gizzards dried and placed in oil, smoked and dried pork, and duck eggs preserved in a mixture of mud and rice chaff, all of which were infected with the bacillus pestis and kept at 37° C.

In rice we found it alive eighteen days after inoculating.

These results correspond with all our other experiments, which plainly prove that the bacillus can not live long on the surface of objects, when dry, at temperatures above 30° C. These results are also in accordance with the clinical and pathological facts, for cases of plague that have their origin in the alimentary tract are comparatively rare, and the spread of the disease has not, as far as I am aware, ever been clearly attributed to food products.

However, tonsillar and intestinal forms of the disease have been recorded, and these cases may be due to the fact that the bacillus can live in milk, butter, and cheese, and similar moist albuminous media, as well as in water (*vide infra*) a long time.

PLAGUE AND WATER.

Plague is certainly not a water-borne disease, although we find that the plague bacillus can live a long time when abundantly inoculated into water containing a trace of organic matter. In one case we kept it alive one hundred and sixteen days, and in another ninety-six days, in water preserved at low temperatures, 17° to 19° C. Under the same conditions the organism lived only six days at 37° C.

In these experiments the water was sterile and abundantly inoculated with a vigorous culture and carefully preserved from contamination in the dark. In nature the water certainly would not be sterile, the effect of sunlight and dilution would play an active part to diminish its vitality and infective power.

THE EFFECT OF TEMPERATURE.

The effect of temperature upon the bacillus pestis is very remarkable. That it is very sensitive to slight changes is well shown in Table A. In fact, temperature seems to be the most important factor in the viability of the organism. It may be kept alive and virulent a very long time in the cold, even though dry, but it can not live long when dry at the temperature of the body. High temperatures, such as 70° C. or more, are invariably fatal in a few minutes. All the published experiments agree upon the influence of temperature upon this microbe. It was this that led some of the early workers to conclude that they were dealing with a frail organism. It is frail when dried at 37° C., but may live for months in the cold. We have never been able to keep it alive more than a few days when dry at 37° C.—three days in flannel, two days in sponge. On the contrary we had little difficulty in keeping it alive on a variety of objects three and four months at 17° to 19° C.

The bacillus is not as sensitive to temperature when kept moist, for under such conditions it will live a very long time in albuminous media at 37° C.

PLAGUE AND COLD WEATHER.

From the experimental studies with the plague bacillus we would infer that the endemic foci of plague should be in cold climates. The organism dies so rapidly when dried at temperatures above 30° C. that we would not expect the disease to be very tenacious in the Tropics. This supposition is only partially borne out by the facts. A certain number of the endemic foci of plague are in a very rigorous climate. Such in particular are the regions of the Garwhal and of Kumaon,¹ where the disease came from the snow-capped Himalayas. In the Transbalkan districts the average temperature in winter is -20° C. In Mongolia the climatic conditions are similar. At Vetlianka during the epidemic of plague in 1878-79 the temperature was -12° C.

PLAGUE IN THE TROPICS.

Netter states in his "*La Peste et son Microbe*" that the disease has never invaded the regions where the temperature is tropical, and he notes its disappearance in Egypt with the summer. This statement would be a confirmation of the fact that the organism does not as a rule live outside of the body very long at temperatures of 37° C. and over.

¹ Netter, "*La Peste et son Microbe*."

But there are other ways of spreading the disease that must be taken into account, such as the direct contagiousness of the pneumonic form of the disease through the coughing, etc., of the plague-laden sputum; also by the means of fleas and rats. This may account for the existence and spread of the disease in such hot countries as the Bombay Presidency, Manila, the Island of Mauritius, Rio de Janeiro, and Santos, all of which are in the Tropics and suffer with tropical heat.

THE EFFECT OF MOISTURE.

Moisture is a definite factor in the viability of the bacillus pestis. The organism must have moisture to grow, and it may remain alive and virulent a very long time in the presence of moisture.

It usually dies quickly when dry. However, this is not invariably the case. We have been able to keep it alive in media such as dried albumin for one hundred and twenty-five days, when it was still virulent for mice. But to be kept alive when dry the organism must be cold, i. e., exposed to a temperature less than 20° C.

In no instance could the organism be kept alive when dry at a temperature of 37° C. for more than a few days.

EFFECT OF SUNLIGHT.

Our experiments confirm those of other workers in this field, who find that for the most part the bacillus pestis soon dies when exposed to bright sunlight. Our work leads us to the conclusion that the heat as well as the sunlight plays an important rôle; also that the effects of the sunlight do not penetrate very deeply. It is therefore safe to say that objects may be efficiently disinfected on the surface by exposing them all day to a bright sun, provided the temperature in the sun is above 30° C.

PLAGUE IN THE SOIL.

The plague bacillus was kept alive a long time in moist garden earth, especially when kept cool. It dies very quickly in dry earth. We were not able to keep it alive longer than twenty-four hours at any temperature in dry earth.

As moist earth will preserve the life of the bacillus it is easy to understand how the infection may live in dirty dwellings. It requires no stretch of the imagination to understand how the infection may be conveyed by the dirt and dust of moist, sunless habitations.

PLAGUE ON NEW MERCHANDISE.

We have not succeeded in keeping plague alive very long when dried upon the surface of objects; even on plush, carpet, paper, wood, sawdust, bone, etc., it usually dies within a few days. In porous sub-

stances such as sponge we found it alive after one hundred and twenty-five days, when allowed to dry, at 19° C. Here again temperature plays an important rôle, for at 37° C., all the other conditions being the same, it lived only two days.

It is therefore very unlikely that new merchandise would be so contaminated as to carry the infection of the disease, especially in hot weather.

PLAGUE IN CLOTHING AND BEDDING.

The bacillus of plague lives long in albuminous matter. Clothing and bedding are especially apt to be contaminated with the discharges from buboes and blisters, sputum, etc. Articles so infected and kept in a cool, moist place could retain the active infective principle a very long time. Our work shows plainly that clothing and bedding may harbor the bacillus of plague for months. In one instance we kept it alive on a piece of crash ninety-seven days; in albumin gelatin balls one hundred and twenty-five days; in sponge, also, one hundred and twenty-five days; in wool fifty-two days.

PLAGUE IN THE MAIL.

According to our results the plague bacillus can not live long in letter mail. In seven tests made with cultures of the organism on paper we found that it usually died within twenty-four hours. At most it kept alive eight days on paper allowed to dry, and fourteen days on paper kept in a moist atmosphere. To live this long it must be kept cool, for, just as in all our other experiments, it died very quickly when dried at the body temperature. We had similar experiences with plague blood upon paper. Letter mail is therefore not apt to carry the infection of plague a long time, especially in warm weather.

VIABILITY AND VIRULENCE.

The bacillus pestis often loses its virulence before it dies. In many of our experiments we found that the time came when the organism grew in bouillon, but lost its pathogenity for animals. This is an important fact from an epidemiologic standpoint, for an attenuated plague bacillus is probably harmless to man, even though its virulence can be increased by artificial means in the laboratory.

GASEOUS DISINFECTANTS FOR THE PLAGUE BACILLUS.

The experiments conducted in this laboratory plainly prove that either sulphur dioxide, when moist, or formaldehyde will kill the bacillus pestis when applied in the strength and methods usually employed for these gases as disinfecting agents. In order to be effective there must be direct contact between the gas and the germ. In other words, these gaseous disinfectants can only be depended upon as surface disinfectants.

In Laboratory Bulletin No. 3, on "Sulphur dioxide as a germicidal agent," Geddings found that 4.25 per cent of that gas for twenty-four hours' time invariably kills the bacillus pestis on the surface.

My own work with formaldehyde (soon to be published) shows that the plague organism is as susceptible to this germicidal agent as other non-sporebearing organisms. Any of the standard methods for evolving the free formaldehyde gas are applicable.

As far as practical disinfection for plague is concerned, it may be mentioned here that sulphur dioxide is probably a much more useful agent for use in ships, stores, houses, and dwellings infested with vermin, because it is destructive to the higher forms of animal life, whereas formaldehyde fails to kill mammals and insects with the same certainty that it kills microbes. In combating plague it is very important to kill the fleas, rats, mice, and other forms of animal life capable of carrying the infection. Sulphur has this power, which formaldehyde totally lacks.

CHEMICAL DISINFECTANTS FOR THE PLAGUE BACILLUS.

The bacillus of plague shows no special peculiarity in its relation to chemical disinfectants in solution. We have found the following ordinary disinfectants to have very marked aseptic powers.

Solutions which prevent the growth of the bacillus pestis.

Bichloride of mercury and hydrochloric acid.....	1 to 50,000
Bichloride of mercury	1 to 25,000
Formalin	1 to 25,000
Carbolic acid (pure).....	1 to 830

The following table gives the results of the German plague commission upon the power of disinfecting agents in relation to the bacillus pestis:

Agent.	Number of minutes necessary.
Carbolic acid:	
5 per cent	1
1 per cent	10
Lysol:	
2.5 per cent	1
1 per cent	5
Sublimate, 1 to 1,000 ..	Immediate destruction.
Chloride of lime, 1 to 1,000	15
Quicklime	30
Milk of lime mixed with equal quantity of salt	60
Black soap:	
1 per cent	More than an hour.
3 per cent	30 minutes.
Sulphuric acid, 1 to 2,000.....	5
Hydrochloric acid, 1 to 1,000	30

THERMAL DEATH POINT.

We had abundant opportunity in our work in making Haffkine prophylactic in demonstrating that a temperature of 70° is invariably fatal in a short time.

The following table shows the maximum number of days we have kept the organism alive on the objects used in these experiments, and the temperatures under which the experiments were conducted. The table shows at a glance how frail an organism the bacillus pestis is at the body temperature. At 22° to 29° C. it shows much more resistance. At 17° to 19° C. it lives a very long time, even when dry.

TABLE A.

Maximum number of days the plague bacillus lived on the various objects at different temperatures.

Temperature.	37° C.	22° to 29° C.	17° to 19° C.
	Days.	Days.	Days.
Gelatin culture in albumin-gelatin balls.....		75	112
Agar suspension in—			
Tap water.....	6	71	116
Water.....	6	71	96
Bouillon culture on—			
Paper, dry.....	0	3	8
Paper, moist.....		8	14
Wood.....	0	0	0
Sawdust.....	0	0	2
Wool.....			52
Flannel.....	3	3	3
Sponge, dry.....	2	97	125
Garden earth, dry.....	1	1	1
Garden earth, moist.....	14	84	93
Bone.....	1	6	17
Agar suspension on bone dust—			
Dry.....	1	4	6
Moist.....	125	108	125
Bouillon culture on crash.....	0	8	97
Cultures on carpet and plush.....	0	0	0
Milk inoculated with plague.....	49	68	32
Bouillon culture in—			
Cheese.....	17	17	13
Butter.....	5	55	72
Cultures on figs and raisins.....	0	0	0
Bouillon culture on—			
Salt beef.....		3	
Orange peel.....		0	
Rice.....	0	3	18
Large variety of Chinese food products.....			3
Spleen of plague rabbit.....	45	1	65
Plague blood on—			
Filter paper.....	0	4	6
Crash.....	0	4	1
Sponge.....	0		9
Wood.....	0		6

No. 1.—PLAGUE CULTURES IN ALBUMIN-GELATIN BALLS.

A gelatin culture of plague was mixed up with about an equal quantity of egg albumin and the mixture soaked on little balls of sterilized absorbent cotton about the size of a pea. These balls soon dried out and shrunk to hard, dry, flaky scales.

One-half was exposed in a Petri dish in the cool chamber at 17° to 19° C., and the other half in a dark room at 20° to 29° C. From time to time one of the dried balls was taken out and planted into bouillon. In case a growth appeared it was tested on media and animals.

Plague cultures on gelatin-albumin balls.

Time.	Cold chamber, 17° to 19° C.	Dark room, 22° to 29° C.
60 days.....	Growth..... Virulent for mice in 3 and 4 days....	Growth. Virulent for mice in 4 and 5 days.
75 days.....	Growth..... Virulent for mouse in 5 days.....	Delayed growth. Virulent for mouse in 2 days.
112 days.....	Growth..... Virulent for white rat in 3 days..... Virulent for mouse in 3 days.....	No growth.
Material expended.		

No. 2.—PLAGUE CULTURE ON ALBUMIN BALLS.

Little balls of sterilized absorbent cotton, about the size of a pea, were saturated with a 4-days-old culture of plague mixed with some egg albumin, as in No. 1, but omitting the gelatin.

Cold chamber, 17° to 19° C.		Remarks.	Dark room, 22° to 29° C.		Remarks.
18 days	+		19 days	+	
22 days	+		22 days	+	
42 days	+		42 days	+	
59 days	+		59 days	—	
68 days	+		68 days	—	
78 days	—		78 days	—	
89 days	—	Contaminated.			Contaminated.

NOTE.—Throughout this paper + means growth and — no growth.

It is plain from these two series, Nos. 1 and 2, that plague will live a long time, even though dry, in an albuminous medium. In the one case it remained alive and virulent one hundred and twelve days, when the material was expended.

The influence of a few degrees of temperature upon the organism is prettily shown in these two series, for it lived thirty-seven days longer in one instance and twenty-six days longer in the other, although all the conditions were exactly the same, excepting a difference of 10° C. in the temperature.

PLAGUE CULTURE IN TAP WATER.

The culture was taken from an agar slant and emulsified in a little bouillon. Four drops of this suspension were added to 15 c. c. of sterile tap water and kept under the conditions as stated below. From time to time a small quantity of the infected water was drawn off and planted for growth, and its virulence tested upon animals.

No. 3.—PLAGUE CULTURE IN TAP WATER, 17° to 19° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
11 days	+	Delayed growth.
17 days	—	Small amount of water used.
20 days	+	2 c. c. used.
25 days	+	Killed mouse in 3 days.
34 days	+	
45 days	+	
66 days	+	Killed mouse in 5 days.
80 days	+	
96 days	+	Failed to kill mouse.
116 days	+	
Material expended.		

No. 4.—PLAGUE CULTURE IN TAP WATER, 22° to 29° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
11 days	+	Delayed growth.
16 days	+	Contamination.
20 days	+	Killed mouse in 24 hours.
25 days	+	
44 days	+	
55 days	+	
71 days	+	Killed mouse in 3 days.
96 days	—	
107 days	—	
Material expended.		

No. 5.—PLAGUE CULTURE IN TAP WATER, 37° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
11 days	—	
17 days	—	
20 days	—	2 c. c. of the water inoculated into bouillon.
24 days	—	

This series (Nos. 3, 4, and 5) of plague in water was repeated, using distilled instead of tap water. But, as the suspensions were made in bouillon, and four drops of bouillon were added to each test tube containing 15 c. c. of water, the results can not be taken as showing the longevity of the bacillus in distilled water.

No. 6.—PLAGUE CULTURE IN WATER, 17° TO 19° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
11 days	+	Delayed growth.
17 days	—	Small amount of water used.
20 days	+	2 c.c. used to inoculate into bouillon.
25 days	+	Killed mouse in 5 days.
34 days	+	
45 days	+	
66 days	+	Killed mouse in 3 days.
80 days	+	
96 days	+	Failed to kill mouse.
116 days	—	Contaminated.
Material expended.		

No. 7.—PLAGUE CULTURE IN WATER, 22° TO 29° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
11 days	—	Contaminated.
16 days	—	Do.
20 days	+	2 c. c. of the water inoculated into bouillon.
25 days	+	Killed a mouse in 9 days.
34 days	+	
45 days	+	Failed to kill mouse.
71 days	+	
96 days	—	
107 days	—	
Material expended.		

No. 8.—PLAGUE CULTURE IN WATER, 37° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
11 days	—	
17 days	—	
20 days	—	
24 days	—	

These results plainly show that the plague bacillus will live a long time in water containing a trace of albuminous matter—there were four drops of bouillon to 15 c. c. of water.

The influence of temperature is very marked in these tests, for the organism lived **only** six days at 37° C., whereas it lived one hundred and sixteen days at 17° to 19° C. It will also be noticed that it lived longer at 17° to 19° than at 22° to 29° C. That is, the cooler the temperature the better does the organism resist.

It is of special importance to notice that the bacillus sometimes lost its virulence in water before it lost its power of vegetation.

PLAGUE ON PAPER.

It was found that plague will not live very long on paper, even when in the presence of moisture. It usually died out in a few days, excepting when kept cool at 17° to 19° C., at which temperature it remained alive eight and fourteen days. It would appear from these experiments that letter mail is not apt to carry the infection, especially in hot weather.

Small slips of white filter paper were inoculated with a three-day-old bouillon culture of the bacillus pestis and placed in a Petri dish under the following conditions:

No. 9.—BOUILLON CULTURE ON PAPER, 37° C.

Time.	Result.	Remarks.
2 days	—	Contaminated with a sarcina.
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

No. 10.—BOUILLON CULTURE ON PAPER, 17° TO 19° C.

Time.	Result.	Remarks.
2 days	—	Contaminated with a long rod.
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

No. 11.—BOUILLON CULTURE ON FILTER PAPER, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	The slips are dry. Delayed growth.
8 days	+	
14 days	—	Contaminated with a mould.
25 days	—	

No. 12.—BOUILLON CULTURE ON FILTER PAPER, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	Delayed growth. Pure culture.
8 days	—	
14 days	—	
25 days	—	
35 days	—	
46 days	—	

In the three following experiments (Nos. 13, 14, and 15) a pledget of cotton soaked with sterile water was kept in the petri dish in order to see the effect of a moist atmosphere. Despite this fact the slips dried out quickly, but the organism lived a little longer than when allowed to dry without the cotton pledget, as in Nos. 9, 10, 11, and 12.

No. 13.—BOUILLON CULTURE ON FILTER PAPER, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	Delayed growth.
8 days	+	The slips are quite dry, despite the cotton pledget soaked with water.
14 days	+	
25 days	—	
35 days	—	
46 days	—	
60 days	—	

No. 14.—BOUILLON CULTURE ON FILTER PAPER, 22° TO 29° C.

Time.	Result.	Remarks.
2 days	+	Killed mouse in 18 hours.
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

No. 15.—BOUILLON CULTURE ON FILTER PAPER, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	
8 days	+	
25 days	—	
35 days	—	
46 days	—	
60 days	—	

PLAGUE ON WOOD.

Plague could not be kept alive on pine wood, as the following tests will show.

Little splinters of white pine were sterilized by heat and soaked in a three-day-old bouillon culture of plague, then placed in petri dishes and exposed under the following conditions:

No. 16.—BOUILLON CULTURE ON PINE WOOD, 17° TO 19° C.

Time.	Result.	Remarks.
2 days	—	
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

No. 17.—BOUILLON CULTURE ON PINE WOOD, 37° C.

Time.	Result.	Remarks.
2 days	—	
4 days	—	
6 days	—	
8 days	—	
12 days	—	
14 days	—	

No. 18.—BOUILLON CULTURE ON PINE WOOD, 22° TO 29° C.

Time.	Result.	Remarks.
2 days	—	
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

PLAGUE CULTURE ON SAWDUST.

Sawdust was sterilized and saturated with a four-day-old culture of plague, and kept in a test tube stopped with cotton, so as to permit drying, under the following conditions:

One tube in the incubator at 37° C.

One tube in the cold chamber at 17° to 19° C.

One tube in a dark room at 22° to 29° C.

No. 19.—SAWDUST, IN THE INCUBATOR, 37° C.

Time.	Result.	Remarks.
2 days	—	
10 days	—	
20 days	—	
31 days	—	

No. 20.—SAWDUST, IN THE COLD ROOM, 17° TO 19° C.

Time.	Result.	Remarks.
2 days	+	
10 days	—	
20 days	—	
30 days	—	

No. 21.—SAWDUST, IN A DARK ROOM, 22° TO 29° C.

Time.	Result.	Remarks.
2 days	—	
10 days	—	
20 days	—	

These results on sawdust correspond with other attempts made to keep the organism alive on wood. In no case was it found alive on wood after a few days.

PLAGUE CULTURE ON WOOL.

Wool was inoculated with a culture of plague and kept in a Petri dish to dry out.

The following is very suggestive, for it indicates that the organism may remain alive a long time in the fur of animals:

No. 22.—PLAGUE ON WOOL.

Time.	Result.	Remarks.
1 days	+	The wool is dry.
7 days	+	
27 days	+	
43 days	+	
52 days	+	
62 days	—	Do.
73 days	—	Do.
93 days	—	Do.

PLAGUE CULTURE ON FLANNEL.

Plague does not live long on flannel.

Small pieces of flannel were sterilized and saturated with a four-day-old bouillon culture of plague and left to dry under various temperatures.

No. 23.—PLAGUE ON FLANNEL, DARK ROOM, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	
9 days	—	
19 days	—	

No. 24.—PLAGUE ON FLANNEL, COLD CHAMBER, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	
8 days	—	
19 days	—	

No. 25.—PLAGUE ON FLANNEL, INCUBATOR, 37° C.

Time.	Result.	Remarks.
3 days	+	
9 days	—	
19 days	—	

PLAGUE ON SPONGE.

Plague was kept alive a long time on sponge, in one instance one hundred and twenty-five days at 17° to 19° C. However, it lost its virulence for mice long before that time. In one instance the organism retained both its vitality and its virulence eighty days.

Small squares of sponge were sterilized and soaked with a three-day bouillon culture of plague. They were allowed to dry under the usual conditions in a Petri dish, as stated in the following tables:

NO. 26.—PLAGUE CULTURE ON SPONGE, 17° TO 19° C.

Time.	Result.	Remarks.
2 days	+	Contaminated.
4 days	+	
6 days ..	+	
8 days	+	
12 days	+	
14 days	+	
21 days	+	
31 days	+	Failed to kill a mouse; pieces of sponge very dry.
43 days	+	
52 days	+	Failed to kill a mouse.
63 days	+	
74 days	+	Failed to kill a mouse.
88 days	+	
98 days	+	
125 days	+	
134 days	—	
144 days	—	

NO. 27.—PLAGUE CULTURE ON SPONGE, 22° TO 29° C.

Time.	Result.	Remarks.
2 days	+	Killed mouse in 9 days of plague.
4 days	+	
6 days	+	
8 days	+	
12 days	+	
14 days	+	
21 days	+	
31 days	+	Contaminated.
43 days	+	
52 days	+	Failed to kill mouse.
63 days	+	
76 days	—	
85 days	—	
95 days	—	

NO. 28.—PLAGUE CULTURE ON SPONGE, 37° C.

Time.	Result.	Remarks.
2 days	+	Contaminated.
4 days	—	
6 days	—	
8 days ..	—	Do.
10 days	—	Do.
12 days	—	
14 days	—	Do.
21 days	—	Do.

These three experiments, as well as the following ones with sponge, show in a very definite manner the effect of temperature on the bacillus pestis. The organism lived only a few days in the incubator at the body temperature, while it survived over four months in the cold— 17° to 19° C.

Another series of infected sponge was exposed in order to verify the rather remarkable results obtained in Nos. 26, 27, and 28. The results obtained were about the same, so it is plain that when well protected in a porous material like sponge the plague bacillus may live and retain its virulence a long time, even when dry.

No. 29.—BOUILLON CULTURE ON SPONGE, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	The pièces of sponge are dry.
8 days	+	
14 days	+	
25 days	+	
35 days	+	
46 days	+	
60 days	+	
70 days	+	0.5 c. c. of a bouillon culture killed mouse in 2 days.
80 days	+	
97 days	—	
106 days	—	
116 days	—	

No. 30.—BOUILLON CULTURE ON SPONGE, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	Killed a mouse in 4 days.
8 days	+	
14 days	+	
25 days	+	
35 days	+	
46 days	+	
60 days	+	Contaminated.
70 days	+	
80 days	+	Failed to kill a mouse.
97 days	+	
106 days	—	
116 days	—	

Another series of experiments with sponge was tried to determine the effects of moisture. A pledget of absorbent cotton saturated with water was kept in the Petri dish along with the test objects. It was found difficult to prevent contamination in the presence of moisture, and also difficult to keep the objects moist so long a time.

No. 31.—BOUILLON CULTURE ON SPONGE, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	Contaminated with a mould.
8 days	+	
14 days	+	
25 days	+	
35 days	+	
46 days	+	Do.
60 days	+	The pieces of sponge are dry.
70 days	+	
80 days	+	Mould contamination.
97 days	+	Do.
106 days	—	
116 days	—	

No. 32.—BOUILLON CULTURE ON SPONGE, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	The sponge is still moist.
8 days	+	
14 days	—	
25 days	—	
35 days	—	
46 days	—	
60 days	—	

PLAGUE ON GARDEN EARTH (ALLOWED TO DRY).

Garden earth was sterilized by heat and well mixed with a young bouillon culture of plague. This mixture was kept in a small bottle, loosely stoppered with a plug of cotton, in order to allow the earth to dry.

Three bottles so prepared were exposed under different conditions of temperature.

The organism did not live long under these conditions.

No. 33.—BOUILLON CULTURE ON DRY GARDEN EARTH, 17° TO 19° C.

Time.	Result.	Remarks.
1 day	+	Still moist.
11 days	—	
17 days	—	
25 days	—	

No. 34.—BOUILLON CULTURE ON DRY GARDEN EARTH, 22° TO 29° C.

Time.	Result.	Remarks.
1 day	+	
6 days	—	
11 days	—	
17 days	—	
25 days	—	

No. 35.—BOUILLON CULTURE ON DRY GARDEN EARTH, 37° C.

Time.	Result.	Remarks.
1 day	+	The garden earth is dry.
4 days	—	
6 days	—	
11 days	—	
17 days	—	
25 days	—	

PLAGUE ON GARDEN EARTH (KEPT MOIST).

The garden earth was sterilized and mixed with a bouillon culture of plague as in the preceding three experiments, the only difference being that the bottles were tightly closed with a cork stopper in order to keep the earth moist.

The bacillus lived a much longer time under these conditions, as was to be expected.

The influence of temperature is well shown here, for at 37° C. the bacillus died after fourteen days, while at cooler temperatures it lived eighty-four and ninety-three days, all the other conditions being precisely similar.

No. 36.—PLAGUE CULTURE ON MOIST GARDEN EARTH, 17° TO 19° C.

Time.	Result.	Remarks.
2 days	+	
11 days	+	
22 days	+	
32 days	+	
46 days	+	
56 days	+	
83 days	+	
93 days	+	
102 days	—	
113 days	—	

Failed to kill a mouse.

Contaminated.

Do.

Contaminated; still moist.

Contaminated; dry.

No. 37.—PLAGUE CULTURE ON MOIST GARDEN EARTH, 22° TO 29° C.

Time.	Result.	Remarks.
2 days	+	Still moist.
11 days	+	
22 days	+	
35 days	+	
47 days	+	
67 days	+	
84 days	+	
93 days	—	
103 days	—	

No. 38.—PLAGUE CULTURE ON MOIST GARDEN EARTH, 37° C.

Time.	Result.	Remarks.
5 days	+	Still moist.
14 days	+	
25 days	—	
36 days	—	
50 days	—	
60 days	—	

PLAGUE CULTURE ON BONE.

Plague will not live long on a piece of dry bone, for a bouillon culture saturated on small porous bits soon died out.

An old dried femur of an ox was broken into bits and sterilized by heat, then saturated with a three-day bouillon culture and exposed in the usual manner in a Petri dish, under different temperature conditions.

No. 39.—BOUILLON CULTURE ON BONE, 17° TO 19° C.

Time.	Result.	Remarks.
1 day	+	Delayed growth; failed to kill mouse.
6 days	+	
11 days	—	
17 days	+	
25 days	—	
34 days	—	
45 days	—	

No. 40.—BOUILLON CULTURE ON BONE, 22° TO 29° C.

Time.	Result.	Remarks.
1 day	+	Bone is dry.
6 days	+	
11 days	—	
17 days	—	
25 days	—	

No. 41.—BOUILLON CULTURE ON BONE, 37° C.

Time.	Result.	Remarks.
1 day	+	The pieces of bone are dry. Contaminated with a mould. Do.
6 days	—	
11 days	—	
17 days	—	

AGAR SUSPENSION ON BONE DUST.

The organism died very quickly when the infected bone dust was permitted to dry, in no case living more than six days. In marked contrast, it lived a very long time on the same bone dust when kept moist.

The bone dust came from the cargo of a vessel from Bombay. It was sterilized by heat and then saturated with an agar-agar suspension of plague in water and thoroughly mixed. The mixture was placed in small bottles and in the following three experiments loosely stoppered with cotton so as to permit drying.

No. 42.—BOUILLON CULTURE ON DRY BONE DUST, 22° TO 29° C.

Time.	Result.	Remarks.
1 day	+	Loosely stoppered so as to dry.
4 days	+	Killed mouse in 3 days.
6 days	—	
11 days	—	
17 days	—	
25 days	—	

No. 43.—BOUILLON CULTURE ON DRY BONE DUST, 17° TO 19° C.

Time.	Result.	Remarks.
1 day	+	Loosely stoppered so as to dry.
4 days	+	The bone dust is dry.
6 days	+	Killed mouse of plague in 18 hours.
11 days	—	
17 days	—	
25 days	—	

No. 44.—BOUILLON CULTURE ON DRY BONE DUST, 37° C.

Time.	Result.	Remarks.
1 day	+	Loosely stoppered so as to dry.
4 days	—	The bone dust is dry.
6 days	—	
11 days	—	
17 days	—	Contaminated with a mold.
25 days	—	

In the following three experiments the bottles were tightly corked, so as to keep the infected bone dust moist. All the other conditions were the same as the three preceding experiments (Nos. 42, 43, and 44).

It is remarkable that the plague bacillus lived so long a time in watery suspension on this material—one hundred and twenty-five days. It is especially to be noted how long the organism lived at 37° C., a temperature which in this work has almost universally proved fatal for the organism, even when kept moist on various objects.

No. 45.—AGAR SUSPENSION ON MOIST BONE DUST, 17° TO 19° C.

Time.	Result.	Remarks.
1 day	+	Kept moist.
3 days	+	
5 days	+	
7 days	+	
13 days	+	
20 days	+	
30 days	+	
43 days	+	Contaminated with a long rod.
52 days	+	
63 days	+	
74 days	+	
88 days	+	Failed to kill a mouse.
98 days	+	
108 days	+	Almost dry.
125 days	+	Dry.
134 days	—	Contaminated.
144 days	—	

No. 46.—AGAR SUSPENSION ON MOIST BONE DUST, 22° TO 29° C.

Time.	Result.	Remarks.
1 day	+	Kept moist.
3 days	+	
5 days	+	
7 days	+	
14 days	+	
31 days	+	Failed to kill a mouse.
43 days	+	
52 days	+	Do.
63 days	+	Mould contamination.
76 days	+	
88 days	+	The bone dust is still moist.
108 days	+	The bone dust is now dry.
125 days	—	
134 days	—	

No. 47.—AGAR SUSPENSION ON MOIST BONE DUST, 37° C.

Time.	Result.	Remarks.
1 day	+	Kept moist.
3 days	+	
5 days	+	
7 days	+	
14 days	+	
21 days	+	
31 days	+	
43 days	+	Killed a mouse in 8 days.
52 days	+	Contaminated with a mold.
63 days	+	Failed to kill a mouse.
74 days	+	
88 days	+	
98 days	+	The bone dust is still moist.
109 days	+	The bone dust is drying out.
125 days	+	The bone dust is dry.
131 days	—	Contaminated.
141 days	—	

PLAGUE ON CRASH.

As a rule, plague dies very quickly on crash, which would indicate that fabrics are not very apt to carry the infection.

Usually it died in a few days, although in one instance it was found to be alive on a piece of crash ninety-seven days from the time it was inoculated. This plainly shows that there are factors in the viability of this organism that are but imperfectly understood which occasionally preserve its vitality.

The small squares of crash were sterilized and saturated with a three-day broth culture of plague and allowed to dry in Petri dishes.

No. 48.—BOUILLON CULTURE ON CRASH, 17° TO 19° C.

Time.	Result.	Remarks.
2 days	—	
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

No. 49.—BOUILLON CULTURE ON CRASH, 22° TO 29° C.

Time.	Result.	Remarks.
2 days	—	
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

No. 50.—BOUILLON CULTURE ON CRASH, 37° C.

Time.	Result.	Remarks.
2 days	—	
4 days	—	
6 days	—	
8 days	—	
10 days	—	
12 days	—	
14 days	—	

No. 51.—BOUILLON CULTURE ON CRASH, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	Contaminated.
8 days	+	
14 days	—	
25 days	—	
35 days	—	
46 days	—	
60 days	—	
		The crash is still moist.

No. 52.—BOUILLON CULTURE ON CRASH, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	—	
5 days	—	
8 days	—	
14 days	—	

No. 53.—BOUILLON CULTURE ON CRASH, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	Mould contamination. Do. Mould contamination. The crash still moist.
8 days	+	
14 days	+	
25 days	—	
35 days	—	
46 days	—	
60 days	—	

No. 54.—BOUILLON CULTURE ON CRASH, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	Permitted to dry in Petri dish.
8 days	+	
14 days	+	
25 days	+	Mould contamination.
35 days	+	Failed to kill mouse.
46 days	+	
60 days	+	
70 days	+	
80 days	+	Contaminated.
97 days	+	
106 days	—	Do.
116 days	—	Do.

PLAGUE ON CARPET AND PLUSH.

Plague could not be made to live on carpet and plush. Many samples of carpet and plush were sterilized and inoculated with plague, but from none was the organism recovered. It is possible that the dye-stuff has something to do with this result, for all the samples used had soluble dyes. Plague could be grown in the solution of the dyes, so that the dyes did not possess the power of preventing growth. It is not evident why plague should not live in carpet and plush as well as it does on crash and paper, and the results emphasize the fact that there are factors that influence the viability of the germ that are not well understood.

PLAGUE IN MILK.

According to all reported works upon the subject, and the three tests that follow, milk is a good medium for plague.

Three test tubes of milk were sterilized and inoculated with a two-day-old agar culture of plague, one loop of culture used for each tube.

The three tubes were incubated at 37° C. for one day, then placed under different conditions:

One left in the incubator at 37° C.

One left in the cold chamber at 17° to 19° C.

One left in a dark room at 22° to 29° C.

From time to time small quantities of the milk were transferred to bouillon for growth. The milk was contaminated, which contamination made the identification of the plague bacillus more difficult and doubtless influenced its longevity.

No. 55.—MILK, IN THE INCUBATOR AT 37° C.

Time.	Result.	Remarks.
9 days	(?)	Contaminated.
12 days	(?)	Do.
22 days	+	Killed mouse of plague in 3 days.
33 days	+	
49 days	+	
58 days	—	Contaminated.
68 days	—	Do.

No. 56.—MILK, IN THE COLD CHAMBER AT 17° TO 19° C.

Time.	Result.	Remarks.
8 days	+	Contaminated.
22 days	+	
32 days	+	Failed to kill mouse.
49 days	—	Contaminated.
58 days	—	Do.
68 days	—	Do.

No. 57.—PLAGUE IN MILK, 22° TO 29° C.

Time.	Result.	Remarks.
9 days	+	
22 days	+	
49 days	+	
58 days	—	No growth of any kind.
68 days	+	Pure and typical plague.
79 days	—	No growth from larger amount of milk inoculated.
99 days	—	

PLAGUE CULTURE IN CHEESE.

The organism lived seventeen days in cheese.

American cheese was melted in a water bath, poured into test tubes, and sterilized by the fractional method at low temperatures.

Four cubic centimeters of a three-day-old growth of plague in bouillon was thoroughly mixed up with 15 c. c. of cheese in each of three tubes. These tubes were then exposed under different conditions of temperature.

- (1) In the incubator at 37° C.
- (2) In the cold chamber at 17° to 19° C.
- (3) In a dark room at 22° to 29° C.

No. 58.—PLAGUE IN CHEESE, IN THE INCUBATOR, 37° C.

Time.	Result.	Remarks.
6 days	+	Growth. Subtilis contamination.
13 days	+	
17 days	+	
26 days	—	
36 days	—	

No. 59.—PLAGUE IN CHEESE, IN THE COLD CHAMBER, 17° TO 19° C.

Time.	Result.	Remarks.
6 days	+	No growth. Do. Do. Do.
13 days	+	
26 days	—	
36 days	—	
47 days	—	
67 days	—	

No. 60.—PLAGUE IN CHEESE, IN A DARK ROOM, 22° TO 29° C.

Time.	Result.	Remarks.
6 days	+	No growth. Contaminated with a spor-bearing rod. Do.
13 days	+	
17 days	+	
26 days	—	
36 days	—	
47 days	—	

PLAGUE IN BUTTER.

Plague may live a long time in butter, especially when kept cool—that is, at 17° to 19° C. The warmer the temperature the shorter the time the organism lives. At 37° C. it remained alive but five days, against seventy-two days at 17° to 19° C.

Butter was melted and sterilized in test tubes at 100° C. for a few minutes each of three days. While liquid it was inoculated with a three-day-old broth culture of plague. Two cubic centimeters of the culture was mixed in each test tube, holding about 15 c. c. of butter.

These tubes were kept under three conditions of temperature:

- (1) In the incubator at 37° C.
- (2) In the cold chamber at 17° to 19° C.
- (3) In a dark room at 22° to 29° C.

From time to time small amounts were taken from each tube and planted in bouillon.

No. 61.—PLAGUE CULTURE IN BUTTER, IN THE INCUBATOR, 37° C.

Time.	Result.	Remarks.
1 day	+	Killed mouse in 5 days.
5 days	+	
14 days	—	
24 days	—	

No. 62.—PLAGUE CULTURE IN BUTTER, IN COLD CHAMBER, 17° TO 19° C.

Time.	Result.	Remarks.
2 days	+	Killed mouse in 3 days.
14 days	+	
24 days	+	
53 days	+	
63 days	+	
72 days	+	
82 days	—	
91 days	—	

No. 63.—PLAGUE CULTURE IN BUTTER, IN A DARK ROOM, 22° TO 29° C.

Time.	Result.	Remarks.
1 day	+	Killed mouse in three days.
5 days	+	
14 days	+	
24 days	+	
35 days	+	
55 days	+	
65 days	—	
71 days	—	

PLAGUE ON FIGS AND RAISINS.

Raisins and figs were contaminated with cultures of plague, and the attempts made from time to time failed to recover the organism by means of cultures and inoculations. This work was undertaken in order to determine whether such articles from infected localities could transmit the infection. The difficulties of determining this question were increased by the fact that it is not practicable to sterilize the figs and raisins. It was also found that both these articles contain 58 per cent of glucose, and that the plague bacillus will not grow in a 58 per cent glucose medium.

The plague organism was never recovered from raisins and figs, even on the day following the inoculation, and it may be definitely stated that there is little danger of these articles carrying the infection.

PLAGUE CULTURE ON SALT BEEF.

Salt beef was sterilized and saturated with a four-day-old broth culture of plague and kept in a dark room at 22° to 29° C.:

No. 64.—BOUILLON CULTURE IN SALT BEEF, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	Contaminated.
18 days	—	No growth.
28 days	—	Subtilis.

PLAGUE CULTURE ON ORANGE PEEL.

Orange peel was sterilized and saturated with a four-day-old broth culture of plague and kept in a dark room at 22° to 29° C.:

No. 65.—BOUILLON CULTURE IN ORANGE PEEL, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	—	
18 days	—	

PLAGUE ON RICE.

Rice was sterilized and inoculated with a four-day-old bouillon culture of plague and kept under the following conditions:

No. 66.—PLAGUE ON RICE, IN DARK ROOM, 22° TO 29° C.

Time.	Result.	Remarks.
3 days	+	
18 days	—	
28 days	—	

No. 67.—PLAGUE ON RICE, COLD CHAMBER, 17° TO 19° C.

Time.	Result.	Remarks.
3 days	+	
18 days	+	
28 days	—	
38 days	—	

No. 68.—PLAGUE ON RICE, IN INCUBATOR, 37° C.

Time.	Result.	Remarks.
3 days	—	
18 days	—	Contaminated.

These results correspond with those of Hankin, who, following different methods, found that plague lived but a short time (few days) on grain.

PLAGUE BLOOD AND TISSUES.

Plague may live a considerable time in the tissues and organs of animals dead of that disease, when these substances are preserved under special conditions. It is probable that the saprophrytes, which soon invade the organism of animals buried or disposed of in the usual way, soon overpower and determine the death of the plague organism. In this work, when the plague bacillus could be protected from such influences, it was found to remain alive and virulent for sixty-five days in a piece of dried spleen.

The spleen of a rabbit dead of experimental plague was placed with aseptic precautions in a petri dish and kept in the cold chamber at 17° to 19° C. The spleen soon dried out. Pieces were taken from time to time and planted in bouillon.

NO. 69.—SPLEEN OF PLAGUE RABBIT.

Time.	Result.	Remarks.
6 days	+	The spleen is very dry.
18 days	+	
20 days	+	
34 days	+	
43 days	+	
65 days	+	Killed mouse in 6 days.
Material expended		

NO. 70.—SPLEEN OF PLAGUE RABBIT.

The spleen of a rabbit dead of experimental plague was placed in a Petri dish, but first the surface was sterilized by moistening and burning with alcohol, and kept in a dark room at 22° to 29° C.

Time.	Result.	Remarks.
1 day	+	
13 days	—	
27 days	—	

No. 71.—SPLEEN OF PLAGUE RABBIT.

The entire spleen of a rabbit dead of experimental plague was kept in a Petri dish in the incubator at 37° C. The spleen soon dried out and became hard and friable. Small pieces were taken from time to time and planted into bouillon.

Time.	Result.	Remarks.
1 day	+	Killed mouse of plague in 18 hours.
12 days	+	
26 days	+	
35 days	+	Contaminated.
45 days	+	Failed to kill mouse.
Material expended.		

PLAGUE BLOOD ON VARIOUS OBJECTS.

Plague-infected blood from animals dead of septicæmic plague was inoculated upon various objects, such as paper, wood, crash, sponge, etc., and kept under various conditions of temperature.

It was found that such thin, dry layers of blood will not preserve the bacillus very long—at most nine days.

No. 72.—PLAGUE BLOOD ON FILTER PAPER.

The heart's blood of a rabbit dead of experimental plague was inoculated on little slips of white filter paper. These slips were placed in a Petri dish and allowed to dry out in a dark room at 22° to 29° C.

Time.	Result.	Remarks.
2 days	+	Killed rabbit over night by intravenous inoculation.
4 days	+	
6 days	—	
13 days	—	
25 days	—	

No. 73.—PLAGUE BLOOD ON CRASH.

The heart's blood of a rabbit dead of experimental plague was inoculated on little squares of sterile fabric (crash) and then allowed to dry out in a Petri dish exposed in a dark room at 22° to 29° C.

Time.	Result.	Remarks.
2 days	+	
4 days	+	
6 days	—	
13 days	—	
25 days	—	

No. 74.—PLAGUE BLOOD ON SPONGE.

Small pieces of sponge were sterilized by heat and impregnated with the heart's blood of a rabbit dead of experimental plague and kept in a Petri dish in the cold room at 17° to 19° C.

Time.	Result.	Remarks.
1 day	+	Contaminated.
6 days	+	
9 days	+	
12 days	—	
18 days	—	
31 days	—	

No. 75.—PLAGUE BLOOD ON CRASH.

Small squares of crash were impregnated with the heart's blood of a rabbit dead of experimental plague and kept in the cold room at 17° to 19° C.

Time.	Result.	Remarks.
1 day	+	Contaminated.
6 days	—	
9 days	—	
12 days	—	
18 days	—	

No. 76.—PLAGUE BLOOD ON PAPER.

Small slips of sterilized white filter paper were impregnated with several drops of the heart's blood of a rabbit dead of experimental plague and exposed in the cold room at 17° to 19° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
9 days	—	
12 days	—	
18 days	—	
31 days	—	

NO. 77.—PLAGUE BLOOD ON WOOD.

Small sticks of white pine were sterilized by heat and soaked in the heart's blood of a rabbit dead of experimental plague and left to dry in a Petri dish in the cold room at 17° to 19° C.

Time.	Result.	Remarks.
1 day	+	
6 days	+	
9 days	—	
12 days	—	
18 days	—	

PLAGUE BLOOD ON CRASH, PAPER, SPONGE, AND WOOD AT 37° C.

Small pieces of crash, white filter paper, sponge, and white pine were sterilized and soaked in the heart's blood of a rabbit dead of experimental plague and kept in the incubator at 37° C.

NO. 78.

Object.	Time.	Result.
Crash.....	5 days	—
Do.....	14 days	—
NO. 79.		
Paper.....	5 days	—
Do.....	14 days	—
NO. 80.		
Sponge	5 days	—
Do.....	14 days	—
NO. 81.		
Wood.....	5 days	—
Do.....	14 days	—

PLAGUE EXPOSED TO SUNLIGHT.

Plague when exposed directly to bright sunlight died quickly. Even in a piece of sponge it could not live an hour in the bright sunlight of a Washington day in the month of May. When exposed more directly on the surface of objects it died within half an hour.

The sun, however, could not penetrate small pieces of plague spleen in five hours.

No. 82.—PLAGUE CULTURES ON VARIOUS OBJECTS EXPOSED TO THE SUNLIGHT.

A two-day-old bouillon culture was reenforced with an oese of an agar culture, and then bits of sterilized paper, crash, sponge, and wood were saturated with the mixture and exposed in a Petri dish to direct sunlight. The temperature in the sun at the time was 33.2° to 35.5° C. This was on May 14, in Washington.

Effects of sunlight.

Object.	Hours.					
	$\frac{1}{2}$	1	2	3	4	6
Filter paper	—	—	—	—	—	—
Crash	—	—	—	—	—	—
Sponge	+	—	—	—	—	—
Wood	—	—	—	—	—	—

No. 83.—SPLEEN OF PLAGUE RABBIT EXPOSED TO SUNLIGHT.

A small piece of the spleen of a rabbit dead of experimental plague was exposed in a Petri dish to bright sunlight, May 26. At the end of five hours it was crushed and planted into broth and gave a typical growth, which growth inoculated into a mouse killed it in three days. The plague organism recovered in pure culture from blood and spleen.

CONCLUSIONS.

(1) The bacillus pestis is not a frail organism. It resembles the hemorrhagic septicæmic group or the cocco-bacilli as far as its viability is concerned.

(2) Temperature is the most important factor in the viability of the plague bacillus. It keeps alive in the cold, under 19° C., a very long time. It dies quickly, especially when dried, at the body temperature, 37° C.

(3) Moisture favors the life of the bacillus pestis. It usually dies in a few days when dry, even in the presence of albuminous matter, provided the temperature is above 30° C. It may keep alive and virulent when dry for months in the cold, under 19° C.

(4) Sunlight kills the organism within a few hours, provided the sun shines directly upon the organism and the temperature in the sun is over 30° C. The effect of sunlight is not very penetrating.

(5) The virulence of the bacillus pestis is often lost before its vegetability.

(6) It is unlikely that new dry merchandise would carry the infection. The organism usually dies in a few days on the surface of objects such as wood, sawdust, bone, paper, etc.

(7) Clothing and bedding can harbor the infection for a long time

and may act as fomites. The bacillus lives for months when dry in albuminous media at temperatures under 20° C.

(8) Food products may carry the infection of plague. The bacillus lives a long time in milk, cheese, and butter. It usually dies quickly on the surface of fruits and prepared foods.

(9) The organism may live a long time in water, although plague is not a water-borne disease.

(10) The plague bacillus does not live long on paper, and first-class mail is therefore not apt to convey the infection.

(11) The colder the climate the greater the danger of conveying the infection on fomites—clothing, bedding, food, merchandise, etc.—and more extensive disinfection is required in such a climate in combating the disease than in tropical regions.

(12) The plague bacillus is destroyed by sulphur fumigation and by formaldehyde gas in the strengths in which these disinfectants are usually employed. The gases can only be depended upon as surface disinfectants. In disinfecting ships, warehouses, dwellings, and other places infested with rats, fleas, and vermin, sulphur is better than formaldehyde, because formaldehyde gas fails to kill the higher forms of animal life.

(13) A temperature of 70° C. continued a short time is invariably fatal for the plague bacillus. The ordinary antiseptics are all efficacious in their usual strength for nonspore-bearing organisms. Efficient surface disinfection may be accomplished by exposing objects all day to the direct sunshine on warm days. The temperature in the sun must be above 30° C.

PLAGUE ON VARIOUS FOOD STUFFS.

[By Passed Assistant Surgeon H. D. Geddings, Marine-Hospital Service.]

JANUARY 11, 1901.

The experiments were continued from the point where Dr. Rosenau's preliminary note left off, and have been the subject of continuous observations in this laboratory for more than a year from that time, having only been brought to a conclusion within the past sixty days.

In general terms it may be said that the further observations have simply confirmed his preliminary views. The growth of the bacillus continued, reaching under various conditions of temperature and moisture to a period of one hundred and twenty-five days on bone dust, where moisture was preserved, and at a temperature of 37° C.; ninety-seven days on crash, temperature 22° to 29° C.; ninety-six days in distilled water, temperature 17° to 19° C.; ninety-seven days in tap water, and other periods too numerous here to mention.

But I would beg here to remind you that while these results are entirely beyond question, and while no effort was spared to make the conditions simulate those naturally obtaining, there were in the end some decided departures from the normal which would obtain under conditions of commerce, trade, etc.

The experiments all showed, too, that under conditions of dryness, exposure to sunlight, and to temperatures above the normal, but not very elevated, the life of the bacillus was short.

Again, too, I would invite your attention to the fact that a condition which obtained in all these experiments was one very far from obtaining under normal conditions, viz, the fact of an intentional, abundant inoculation with a pure culture of the materials subjected to test, and this culture one of known activity and virulence.

A second departure from normal conditions was in the fact that all the materials receiving the abundant inoculation of the virulent pure culture were sterile. In other words, the plague bacillus was not only put to growing under the before-mentioned favorable conditions of heat and moisture, but was relieved in toto from that microbial symbiosis which, in the economy of nature, plays so important a part in the suppression of pathogenic organisms.

I do not think, therefore, that the laboratory experiments, complete and painstaking though they be, should be held as establishing a standard in the case of food products where the following conditions would establish a strong dissimilarity of conditions:

First. Food products are never intentionally inoculated with plague organisms, or, for that matter, with any others. On the contrary, no matter how careless the operator and how slovenly the method of preparation, care is exercised to protect from gross contamination. The product, it is true, may accidentally become contaminated by preparation in a dwelling where plague prevails. After preparation it may become contaminated in the warehouse or store by being "muzzled" by a rat suffering from the pneumonic form of the disease, or a plague-infected rat may nest or even die on the products, or the foods may be contaminated by dust containing virulent and viable plague bacilli.

But granting any or all of these possibilities, should the contamination take place it is at once confronted by the following condition:

Second. That all products for wear, for use in the arts, or for food are the seat of contamination by saprophytic bacteria, and that there is at once set up a conflict between the pathogene on the one hand and the saprophyte on the other, in which contests the hardier saprophyte usually comes off victorious over the more deadly but more highly organized and sensitive pathogene.

I repeat, therefore, that the conditions obtaining in exact laboratory experimentation are not applicable to and do not form an invariable rule of conduct for quarantine matters, in which more normal natural conditions obtain.

The continuation of the experiments which formed the basis for Dr. Rosenau's preliminary note lead in the main to the conclusion that if a substance is favorable for the retention of moisture, and a comparatively low temperature (17° to 19° C.), it will afford a good culture medium for the growth of the plague bacillus, unless it have in itself some inherent property which renders it unsuitable. This latter may be something added to it in the process of manufacture, as the sizing or filling in certain fabrics, or the resins or gums contained in woods, or the bacteria which find a habitat in manufactured foods.

Thus the plague bacillus failed to grow on linen crash until it had been thoroughly freed of its sizing by washing; on sawdust it lived but one day; on flannel only three days; on a piece of carpet (the effect probably of the dye) it did not grow at all.

The following food products were tried, and gave the following results: On cheese it lived from thirteen to seventeen days, but this only after the cheese had been thoroughly sterilized and melted; on rice, sterilized, three days at 22° to 29° C.; on dried salt beef, three

days; on orange peel, no growth; on figs and raisins, no growth, though subsequent experiments showed this to be due to the amount of glucose or fruit sugar contained.

Further, a staple article of Chinese food, a duck dried and smoked, was obtained, and from this small pieces were cut and placed in sterile Petri dishes. These pieces were then liberally inoculated with a bouillon culture of the plague bacillus and the dishes placed in the incubator. Another portion was placed directly in a tube of bouillon, and its growth proved that it was free from bacteria, save the hay bacillus and its spores.

For several days small pieces were cut off from those contained in the dishes, and these were also planted into bouillon tubes. The length of life of the plague bacillus under these conditions and this microbial association is uncertain. For two or three days they were present in numbers; this number grew rapidly more limited, and by the twelfth day the plague bacilli were difficult of detection. Between this and the eighteenth day they disappeared completely, though the hay bacillus was still active and grew in great abundance.

Subsequently a large assortment of Chinese food products was obtained, and with these experiments were made. The products in question consisted of the legs of smoked and dried ducks; dried oysters; dried cuttlefish; dried ducks' gizzards; ducks' gizzards dried and then placed in oil; smoked and dried pork; duck eggs preserved in a mixture of mud and rice chaff. In this last the mixture had originally been wet, but had dried out until there only remained a mass of pulverulent earth and chaff surrounding the egg—in other words, dry earth.

Portions from each of these specimens were thoroughly inoculated with a bouillon culture of the plague bacillus, and the dishes containing them were placed in the incubator at 37° C. for about twenty-eight hours. Small pieces were then cut off each and these pieces planted into bouillon tubes, which were incubated for twenty-four hours.

Of the seven specimens, three alone showed any growth viz. the pork, the dried gizzard, and the cuttlefish.

A specimen from the growth in such case was removed and stained with carbol-thionin solution (the stain by election for plague bacilli), and microscopic examination showed the following: On the pork the growth was the *B. subtilis* (hay bacillus) alone; on the dried gizzard, hay bacillus; on the dried cuttlefish, hay bacillus and an ordinary mold.

In view of these results, therefore, I would respectfully submit the opinion that it is unlikely that the Chinese food products in question could convey the germ of plague, even should they accidentally become contaminated with the same, and under the ordinary conditions of com-

mercial intercourse I much question whether such contamination is likely to occur, or whether in the presence of the temperature obtaining in the holds of ships the organism would have any prolonged life.

As these results may have a bearing on the quarantine restrictions at present in force against these food stuffs, they are submitted at once, without waiting for further observations, which, however, will be carried on from time to time.



BULLETIN No. 5 OF THE HYGIENIC LABORATORY,

Treasury Department, U. S. Marine-Hospital Service,

WALTER WYMAN, *Surgeon-General.*

AN INVESTIGATION

OF A

PATHOGENIC MICROBE

(B. typhi murium—Danyz)

APPLIED TO THE

DESTRUCTION OF RATS.

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AN INVESTIGATION OF A PATHOGENIC MICROBE

(*B. typhi murium*—Danyz)

APPLIED TO THE DESTRUCTION OF RATS.

[M. J. Rosenau, Passed Assistant Surgeon and Director of the Hygienic Laboratory,
United States Marine-Hospital Service.]

The subject of the destruction of rats has assumed great importance within recent times on account of the spread of plague to the four quarters of the globe.

In the short time since 1894, when plague broke from its Eastern confines where it had slumbered so many years, a great mass of exact scientific knowledge has been gathered concerning the disease. The relation of the rat to the spread of the disease has especially engaged the attention of investigators.

During the great outbreaks of plague in the middle ages the unusual mortality among the rats was noticed, and it was obvious then that these animals played some rôle in the spread of the disease. We now know definitely that the rat is susceptible to plague. This rodent sickens and dies of the disease in much the same manner as man. An epidemic may be foretold by a great increase of mortality in rats, for when this occurs it may be shown that the rats first contract the disease and afterwards transmit it to man.

The destruction of rats has, therefore, become of the first importance from the standpoint of the public health and the prevention of the spread of plague.

Plague has threatened our country from both its seacoasts, and it is probable that the only reason it has not spread in San Francisco is due to the fact that the disease has not been prevalent among the rats.

In the spring of 1900, J. Danyz described a new method for the destruction of rats by means of cultures of a certain bacillus (*a*). This bacillus he obtained from a spontaneous epidemic among harvest mice and by means of rather complicated and artificial methods he managed to increase its virulence so that it became pathogenic for the several species of rats. The claim is made that the employment of the cultures

a Annales de l'Institut Pasteur, April, 1900. A translation of the article by Danyz appeared in the PUBLIC HEALTH REPORTS of May 25, 1900, Vol. XV, No. 21.

of this bacillus, sold under the name of "Rat Virus," is efficient for the destruction of these rodents.

The following work was undertaken in order to test the validity of this claim.

THE ORIGIN OF THE VIRUS.

In 1889 Loeffler discovered the *bacillus typhi murium*, which he isolated from a spontaneous epidemic among white mice, and which he applied with success to the destruction of harvest mice (*m. arvicola*). Other bacteriologists have observed similar epidemics and have isolated the microbes thereof, morphologically identical with the bacillus of Loeffler, but more or less virulent for the several genera and species of the little rodent.

For example : The *B. typhi murium* of Loeffler was only frankly pathogenic for mice (*m. musculus*) and for harvest mice (*m. arvicola*): A bacillus isolated by Laser was pathogenic for the *m. agrarius*, that isolated by Merechkowski for the *spermophiles*, and finally that isolated by Issatchenko for white rats.

Each of these various bacilli is of such variable virulence that it can not be used practically for the destruction of all species of these rodents.

Danyz, therefore, conceived the notion that it would be of great interest, first to extend the field of action of one of these organisms by increasing its virulence so that it would attack other species of rodents and then, this virulence increased, to maintain it at its highest point. This is how he proceeded to solve this problem.

He first isolated a bacillus from a spontaneous epidemic among harvest mice. This organism was a cocco-bacillus, presenting in general the characteristics of the colon bacillus, and resembling the bacillus of Loeffler—*B. typhi murium*. From the first this bacillus showed a slight pathogenicity for gray rats (*m. decumanus*). Out of 10 animals fed with a culture of this microbe 2 or 3 would die; several others would sicken and recover; others still appeared completely refractory. The fact that a certain number of the rats fed with these cultures always succumbed led to the hope that it would be possible to increase the virulence of this particular microbe by the generally accepted methods—that is to say, by a certain number of passages from rat to rat.

Danyz first tried to increase the virulence of the organism by this means, but he found that successive passages from rat to rat, whether by feeding or by subcutaneous injection, ended by enfeebling rather than increasing the virulence of the microbe. He found that it was rarely possible to go beyond 10 to 12 passages. Sometimes the series was stopped at the fifth passage, or even sooner, by the survival of all the animals undergoing experiment. The result was exactly the same, if, instead of alternating each passage through the animal by a culture

in bouillon or agar, the bodies of animals dead of a preceding passage were fed to others.

It was, therefore, plain that in the evolution of an epidemic caused by this microbe it was necessary to take account of the indisputable diminution of the virulence of the microbe, as well as the natural resistance of the survivors.

Finally, passages of cultures in collodion sacks, inclosed in the peritoneal cavities of rats, were tried, both in interrupted series and by alternating each sack culture with a culture in bouillon or on agar, but the end was invariably a notable diminution of virulence when administered by the digestive tract.

Danyz finally managed to increase the virulence of the organism, so that it was pathogenic for rats, by the following process:

DANYZ'S METHOD FOR INCREASING AND MAINTAINING THE VIRULENCE OF THE VIRUS.

A culture of the bacillus was selected that was fatal for mice in four to five days, and grown in bouillon to accustom it to an anaërobic existence. This was accomplished by growing the culture in flasks as completely filled as possible. The flasks were placed in the incubator until the culture developed, and then kept at ordinary temperature until a deposit formed and the bouillon became perfectly clear. This may take four or five days, and its object is, as above stated, to accustom the microbe to an anaërobic existence.

From the flasks the culture was passed in a collodion sack, which is kept from twenty-four to thirty-six hours in the abdominal cavity of a rat, and then planted anew in ordinary bouillon and thence again into flasks. The culture was transferred from these last flasks to agar, and it is these cultures on agar that Danyz gave to mice to eat, after having diluted them with water and soaked bread and grain in the dilution.

This series of operations was repeated several times, and at the fourth or fifth repetition a decided increase in virulence was noted. Mice, which died only at the end of four to seven days, now died in thirty-six to sixty hours after the ingestion.

After this decided increase in the virulence for mice was obtained, the mice were replaced by white rats, commencing with young rats a month or six weeks old and, as the passages are continued, taking older rats. Proceeding thus and making collodion sack cultures in the abdominal cavity of the species of the animal which it was desired to infect, the culture was specialized, so that it was rendered sufficiently virulent in ten passages. Operating in this manner, Danyz finally succeeded in rendering regularly virulent for gray rats (*m. decumanus*), then for black rats (*m. ratus*), and finally for white rats, a culture which was originally but slightly virulent for the gray rat and entirely innocuous for the other two.

The bouillon used was made from horse meat with 1 per cent peptone, and to which was added a little carbonate of lime to neutralize the acids which are formed during culture and which rapidly diminish the virulence of the microbe.

After this long and painstaking procedure, Danyz obtained a very virulent culture that, contained in flasks and kept from the influence of light and air, preserved its virulence for several months. Planted on agar it preserved its virulence without appreciable diminution for two months. In bouillon, in flasks or tubes stoppered with cotton, it altered very rapidly.

One can not read the foregoing account without admiration for the patience and perseverance required to increase the virulence of an organism by so many passages through the peritoneal cavities of rats by means of the collodion sack method.

DESCRIPTION OF THE ORGANISM.

The cultures with which I did the following work were obtained from the Pasteur Institute.

The organism is a cocco-bacillus, showing distinct motility. Stains well by the ordinary stains and does not stain by Gram's method.

It grows well at ordinary room temperature; also in the incubator, and on all the ordinary media. In bouillon it produces a uniform cloudiness in twenty-four hours. A slight scum forms after several days' growth, which falls to the bottom when shaken. In Dunham's solution it grows well, but produces no indol in twenty-four hours' growth.

It turns blue litmus milk red, but does not cause coagulation.

It grows the whole length of the stab in gelatin, forming small whitish colonies in the deeper portions of the tube. It does not grow over the entire surface of the gelatin tubes. Does not liquify.

It grows under anaërobic conditions.

It ferments glucose bouillon, but not lactose bouillon.

In glucose bouillon it produces 1-CO₂, 5-H.

It also produces H₂S.

From a general biological standpoint it is plain that this bacillus belongs to the para colon group, and is very similar to the bacillus of rabbit septicæmia, hog cholera, and icteroides—as far as its morphological and cultural characteristics are concerned.

VIRULENCE OF THE VIRUS FOR RATS.

One hundred and fifteen rats were fed with the cultures in various ways during the course of my experiments with the virus. Of these, 46 died—less than half.

Most of the rats used were the gray rat (*m. decumanus*) and the tame white rat. A few (8) of the wild brown, or Norway rat, were used.

The virus is in reality pathogenic for these three kinds of rats when ingested. No special difference was noted in its effects upon the various species.

As the work progressed it soon became evident to me that the result depended largely upon the amount of the culture ingested. By starving rats for a day or two and then giving them all they could be induced to eat and drink of the cultures, a very positive result was obtained. In one instance of 27 rats so fed, all died within a week. If the rats are given a small amount the effect is uncertain—only a few die. In one instance I fed 70 rats with 4 agar tubes, and only 7 died. Upon refeeding them with very large quantities, 9 more died. The survivors were then fed with all they could be induced to eat every day for a week, without effect.

It, therefore, seems plain that a large primary dose proves fatal, and a small dose is not only uncertain, but produces an immunity. This is a very important factor, for it is likely that in the wild state rats would often partake of an amount too small to cause death. Such rats may then subsequently eat large amounts of the culture with impunity.

It would seem, then, that after all, the virus is not so different from the laying out of a chemical poison, depending as it does for its effect upon the amount ingested. A chemical poison, however, does not possess the disadvantage of producing an immunity. Another disadvantage possessed by the virus is the rapid deterioration in virulence which occurs when it is exposed to the action of air and light, or when it becomes dry, as is very apt to happen when laid out for rats in the wild state.

Against this the virus has the very decided advantage over the usual chemical agents used to kill rats that in so far as known it is harmless for man and domestic animals.

THE DISEASE PRODUCED BY THE VIRUS HAS FEEBLE POWERS OF PROPAGATING ITSELF AMONG RATS.

Danyz has pointed out the fact that the epidemic produced by his virus in rats is self limiting. This is due to two causes:

1. The attenuation of the virus.
2. The resistance of the rats.

That the disease can be spread from rat to rat by eating each other can not be doubted, but this method of the spread of the infection is very feeble. I fed rats upon the organs (spleen, liver, etc.), of other rats dead of the infection, without producing any effect. Rats will sometimes eat each other, but sometimes they refuse. I fed dead and sick rats to 10 healthy rats in a cage, and although food was withheld for four days they refused to touch the dead bodies. On other occasions the rats were very vicious, fighting and killing the sick and feeble and eating the dead bodies.

As will be seen from the details of the work which follow, I failed to propagate the disease from rat to rat.

This feeble power of the infection to spread from rat to rat is a decided limitation to the virus as far as its practical application is concerned. It is easy enough to make the rats in a cage eat the cultures soaked in food, but in a wild state this would not be so easy, and if only the rats that eat the virus die, there is little advantage over phosphorous, arsenic, and other chemical poisons.

It is not considered necessary to give in full detail all the experiments made with the virus. A few of the more striking ones follow.

NO. 1. SEVENTY RATS FED ON CULTURES IN INCREASING AMOUNTS.

Seventy white and gray rats were fed April 8 with 4 agar slants (original) of Danyz's virus No. 2, obtained direct from the Pasteur Institute. The cultures were shaken up with water and soaked on dry bread, according to the instructions. The agar itself was also fed to the rats. They ate it all up greedily, food having been withheld for a day previously—

	Died.
Twelve days after feeding.....	1
Fourteen days after feeding.....	2
Thirty days after feeding.....	1
Thirty-one days after feeding.....	1
Thirty-two days after feeding.....	1
Thirty-three days after feeding	1
Total.....	7

Seven of the 70 rats died. One of the 7 died of a peritonitis, due to a long nonmotile bacillus which had no resemblance to the organism that was fed to the rats. The others died of a disease evidently due to the ingestion of the cultures.

In most of the animals the small intestines were found to be the seat of inflammation with enlargement of the lymphatic glands. The spleen and liver showed little change. The lungs were often the seat of congestion and hemorrhagic inflammation—sometimes infarct. The ingestion of the virus causes a local reaction in the intestines, followed by an invasion of the organism into the blood, producing a septicæmia.

The surviving rats, remaining perfectly well to all appearances, were again fed with a fresh culture of the virus. They were now divided into 4 lots and fed with variable amounts in order to determine whether the small quantity ingested produced an immunity and whether the effect is proportionate to the amount eaten.

THIRTY-THREE RATS REFED WITH LARGE QUANTITIES OF BOUILLON CULTURES.

On May 16, after two days' starving, 33 of the surviving rats of experiment No. 1 were given a liter of a young bouillon culture. One-half of this was given as drink, the other half soaked on dry bread. The rats

ate and drank most of it greedily. On May 17 and May 18 this was repeated. No other food was allowed; so that in all the 33 rats ingested almost 3 liters of the virus in three days.

	Died.
May 20, four days after feeding.....	4
May 21, five days after feeding.....	4
May 22, six days after feeding.....	1
Total.....	9

Nine of the 33 rats died. Death came rather suddenly. The others remained apparently healthy. An increased desire to drink probably indicated a feverish condition in some of them. Ordinarily rats die rather slowly from the effects of this virus. They have fever, show symptoms of distress, they are weak and prostrated, and refuse food for a day or two before death.

It, therefore, seems plain that the ingestion of a small amount of the virus killed a few of the susceptible rats—7 out of 70, and left the survivors with a considerable protection, for, after feeding them for three days with overpowering doses, only 9 died out of 33.

TEN RATS REFED ON 10 c. c. OF BOUILLON CULTURE.

Ten of the rats that resisted experiment No. 1 were separated in a cage and subsequently fed with 10 c. c. of a young bouillon culture soaked on bread. This produced no effect.

TEN RATS REFED ON 2 AGAR SLANTS.

Ten of the rats that resisted experiment No. 1 were subsequently fed with 2 agar slants, shaken in water and soaked on bread in accordance with instructions of Danyz. This produced no effect.

TEN RATS GIVEN DEAD RATS TO EAT.

Ten of the rats that resisted experiment No. 1 were placed in a separate cage and deprived of all food for four days, during which time rats sick and dead of the infection were placed in the same cage. They refused to touch the dead bodies. None of the 10 rats took the disease.

It is evident from the foregoing experiments that small primary doses are uncertain in their effects.

Another test was made, using large overpowering doses from the start.

NO. 2. TWENTY-SEVEN RATS FED ON VERY LARGE QUANTITIES OF CULTURE.

Twenty-seven white and gray rats were deprived of food for twenty-four hours and then given (April 11) a liter of a young bouillon culture of the virus soaked on bread. In addition they were given another

liter of the culture as drink, and 6 agar slants. They ate most of the bread during the night, and drank a considerable quantity of the liquid. Two days following (April 13) they were given another liter of a young culture soaked on bread. On this day the rats seemed sick, 1 died. They ate little or none of the bread, but licked up all the liquid. On April 14, the third day, the rats were sick, very thirsty, refused food, 2 were dead. From this on, the rats refused food and succumbed one after another until all were dead—

	Died.
Two days after feeding.....	1
Three days after feeding	2
Four days after feeding.....	10
Five days after feeding.....	7
Six days after feeding.....	7
Total	27

All these 27 rats, then, died within a week after being fed with an overpowering amount of the virus.

The result, therefore, seems to depend on the amount ingested as well as the virulence of the cultures.

RATS AND MICE FED ON TOXINS.

A toxin was made by growing the organism in flasks containing 1 per cent peptone bouillon. The flasks were left over night in the incubator at 37° C., and then the growth continued at room temperature. At the end of two weeks the cultures were killed by exposing the flasks to 70° C. for several hours. The resulting product was fed to mice and rats, and was found to produce a certain toxic effect. It seems very probable that the toxin favors the pathogenic action of the organism. This question, not having a direct practical bearing on this particular subject, will be discussed at another time. It may be stated here, however, that animals fed on the toxin seem to acquire a certain amount of immunity against the virulent cultures.

VARIOUS METHODS OF FEEDING.

Rats were fed upon the organs (livers and spleens) of other rats dead of the infection, and in other ways, without producing any effect.

THE SURVIVORS FED ON LARGE QUANTITIES.

Finally, I had about 60 rats, which had survived the various experiments, on my hands. I fed them all with large amounts of cultures in order to get rid of them. They were given as much of the cultures as they could be induced to eat, every day for a week, without apparent effect. The immunity produced is, therefore, very positive and lasts at least several months.

SUMMARY.

The substance known as Danyz's Virus consists of a culture of a bacillus belonging to the para colon group. It appears to be indentical with the *bacillus typhi murium* of Loeffler.

This organism is naturally pathogenic for mice, in which rodents it sometimes produces spontaneous epizootics. Its virulence has been raised and specialized by artificial means in the laboratory, so that it has become fatal for rats by ingestion. This artificial virulence is not very stable. It may be maintained under special conditions a few months, but the virulence is apt to fall off, especially on exposure to light and air.

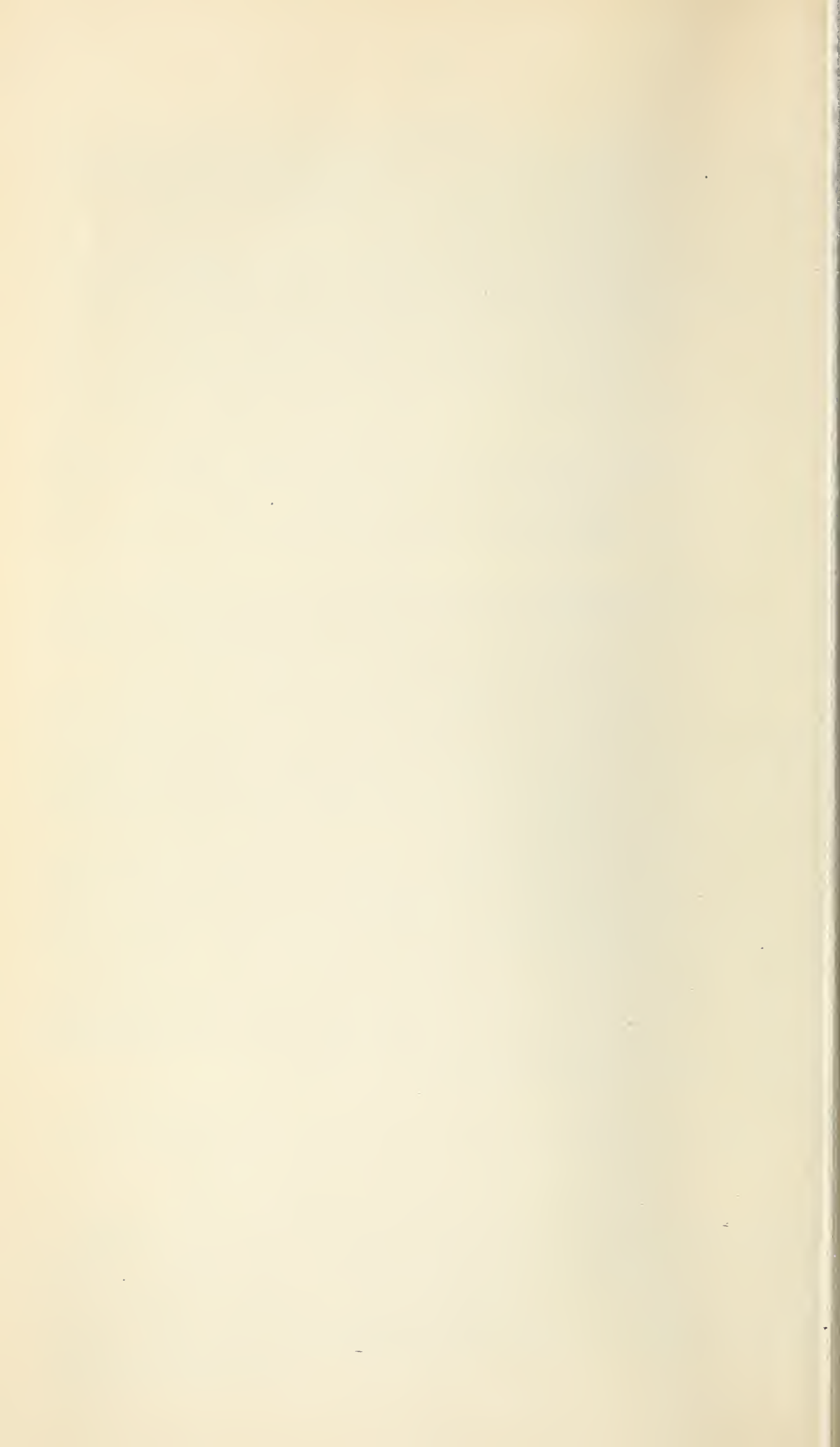
As far as rats are concerned, the effect depends somewhat on the amount ingested. Large amounts are frankly fatal. Small quantities are uncertain. Rats that survive the ingestion of the virus are rendered immune. Such rats may eat large amounts of the virulent virus with impunity.

The infection caused in rats by eating the virus has feeble power of propagating itself from rat to rat. It, therefore, can not produce a widespread epizootic among these rodents. In practical use it must be spread around so that as many of the rats as possible will eat it.

In many respects it resembles a chemical poison, with this great advantage, viz, that it is harmless, in so far as known, to man and domestic animals. It has the great disadvantage, that chemical poisons do not possess, of rendering the animals immune by the ingestion of amounts that are insufficient to kill, or by the ingestion of cultures that have lost a little of their virulence.

In my experiments I succeeded in killing less than half the number (46 out of 115) of rats fed. The conditions in a cage are so much more favorable for the fatal action of the virus than could possibly be the case in nature that it is safe to assert that a less number would succumb in a wild state.

The virus may, therefore, be used as one of the means in the fight against rats, but it is far from being a sure means of exterminating these rodents in a particular place.



BULLETIN No. 6 OF THE HYGIENIC LABORATORY,

Treasury Department, U. S. Marine-Hospital Service,

WALTER WYMAN, *Surgeon-General.*

SEPTEMBER, 1901.

Disinfection Against Mosquitoes

WITH

FORMALDEHYD AND SULPHUR DIOXID.

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DISINFECTION AGAINST MOSQUITOES WITH FORMALDEHYD AND SULPHUR DIOXID.

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U. S. Marine-Hospital Service.]

Until lately, mosquitoes and flies were looked upon merely as annoyances, but since it has been proved that they are able to transmit the infection of pestilential diseases, we must now regard them as dangerous vermin. When the matter is generally understood, it will be a greater reproach to the housewife to have mosquitoes and flies in the home than bed bugs, and it is the duty of sanitarians to spread an abhorrence for these most common and most dangerous of domestic pests.

The mosquito is known to transmit the infection of malaria and filariasis. The theory of Finlay, so brilliantly proven by Reed and his colleagues (Carroll, Lazear, and Agramonte) of the Army Medical Commission, that the mosquito transmits yellow fever must now be accepted as an established fact. One of the next problems is the destruction of the infected mosquitoes, and we have undertaken the following work to test the insecticidal value of formaldehyd gas and sulphur dioxid in the work of practical disinfection :

METHODS.

In our experiments we have endeavored to imitate the conditions found in actual practice in so far as they were compatible with accurate control of the results.

The mosquitoes used were good specimens of the *Culex pungens* raised in our laboratory. The insects were from one to seven days old and consisted of males and females in about equal proportions. They were exposed in battery jars covered with gauze, or in small pill boxes with gauze lids. The battery jars were used in order to allow the mosquitoes to seek and choose their own protection. To this end, crumpled crash toweling and paper, sometimes dry, sometimes moist, were placed loosely in the jars. It was always found that the insects would hide in the more remote folds and corners, thus often entirely escaping the stronger percentages of the gas.

After this self-protecting instinct was clearly demonstrated, mosquitoes were placed in pill boxes, as with these we were able to expose the insects

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under definite conditions, and to determine exactly the amount of protection required to prevent the penetration of the gas to the mosquitoes in sufficient strength to kill them.

The pill boxes were small cylindrical cardboard boxes, one-half an inch high by one and one-half inches in diameter. The lid of each box was replaced with gauze mosquito netting.

Mosquitoes thus confined often die within twenty-four hours under favorable conditions of temperature and moisture. Therefore, if one survives in a box containing several insects, the death of the others must not be attributed to the effects of the disinfecting gas. All the mosquitoes in the box must be dead to draw positive conclusions.

Another advantage of these boxes was that we were enabled to compare the germicidal with the insecticidal power of the gases used. Cultures of bacteria were exposed upon slips of paper in similar pill boxes, which were sterilized for this purpose. These boxes containing the cultures were placed alongside the boxes containing the mosquitoes, so that the two were subjected to exactly similar conditions as far as that was possible.

The cultures were exposed upon sterile strips of white filter paper. The spores of the hay bacillus and of anthrax were thoroughly dried in the incubator at 37° C. Rich garden earth was emulsified with water and the strips impregnated with the suspension. These were always dried before being exposed. Fresh young cultures of a staphylococcus, pyocyaneus, and bacillus icteroides were used to inoculate the slips and were exposed moist and freshly prepared. These were selected as representative of the non-spore-bearing group of microorganisms.

In some of the experiments the bacteria and the mosquitoes were not exposed under precisely similar conditions. In such cases, noted hereafter, the slips of paper impregnated with the cultures were placed in Petri dishes, in envelopes, and in small boxes. The Petri dishes were opened so that the gas could gain free access to the test slips. The envelopes were not sealed. The boxes were small cardboard cylinders about 2 inches long by 1 inch in diameter, with 5 small holes punched in both top and bottom to permit the entrance of the gas.

FORMALDEHYD GAS.

It is a well-known fact that formaldehyd gas readily enters into combination with the protoplasm of the lower forms of vegetable life, which makes it a very efficient germicide. It is, however, not toxic to the higher forms of animal life. It is very irritating in its effect upon the mucous membranes of rats, mice, guinea pigs, rabbits, and mammalian animals generally, but not necessarily fatal, even after prolonged exposures. Many insects, such as roaches and the like, may be exposed to strong concentrations of the gas a long time without apparent effect.

Formaldehyd gas kills mosquitoes whenever the gas comes in direct contact with them in sufficient concentration and for a sufficient length of time. When exposed *directly* to the gas produced by any of the methods commonly used for disinfecting purposes, the mosquitoes die within few minutes. If the insects are confined in a bell jar and some formalin is dropped inside, they soon show signs of agitation and shortly drop down, dead. They may, however, live over night in a very feeble atmosphere of the gas.

The conditions necessary to obtain this direct contact, however, can not always be obtained in actual practice. Rooms are frequently not tight enough to obtain the concentration of the gas required. The mosquitoes can not be held in direct contact with the gas, for their sense of self-preservation helps them to escape. The period of irritation, lasting several minutes even in the bell jar, enables the insects to hide in available places, such as the folds of garments, hangings, or fabrics, or in the cracks and crevices where the gas only reaches in a diluted form. If the room is not thoroughly sealed, some of the mosquitoes will surely get away, for their instinct in finding tiny avenues of escape is remarkable. The escape of one infected mosquito might be the spark that would rekindle an epidemic.

In general, it may be stated that to succeed in killing mosquitoes in a closed space with formaldehyd gas, the following definite requirements are essential. A large volume of the gas must be liberated quickly, so that it may diffuse to all portions of the room in sufficient concentration. The room must not have cracks and chinks where the insects will breathe the fresh air entering, especially if these openings are to windward. The room must not have heavy drapery, clothing, bedding, or other fabrics, so disposed that the insects may hide in the folds, away from the full effects of the gas.

Four methods of evolving formaldehyd gas were used, namely :

1. The sheet method.
2. The Kuhn lamp.
3. The Trenner-Lee formaldehyd disinfector.
4. The autoclave.

THE SHEET METHOD.

This method was applied by hanging sheets in the room and spraying formalin (40 per cent) upon them by means of Dr. Behn's formaldehyd sprinkler. The solution was sprinkled on the sheet in small drops in order to facilitate evaporation and prevent polymerization. For bacterial disinfection, 5 ounces of the solution are prescribed for each 1,000 cubic feet of space, the time of exposure to be not less than five hours.

No. 1.

In a room of 2,000 cubic feet capacity, 4 sheets were hung on cords crossing the room. Twenty ounces (600 c. c.) of formalin (40 per cent)

were sprayed on the sheets and the room was closed. This room has 2 doors and 4 windows, is of wooden construction, and rather leaky. The windows and doors were simply closed and no pasting or calking was done. Time of exposure, six hours.

Mosquitoes.

	Dead.	Alive.
In battery jar one-third full of absorbent cotton and crumpled toweling..	0	12
In pill boxes—		
1. In single fold of a towel.....	0	3
2. In double fold of a towel.....	0	2
3. Rolled loosely in a towel.....	0	3
In large test tubes—		
1. Very thin layer cotton tied over mouth.....	1	0
2. Very thin cotton stopper.....	0	1
3. Ordinary cotton stopper, 1½ inches.....	0	1
4. Large cotton stopper, 2½ inches.....	0	1

In order to compare the effect upon mosquitoes with the germicidal power of the gas, bacteria were exposed at the same time as follows:

	Garden earth.	Subtilis spores.	Anthrax spores.	Staphylococcus.	Pyocyanus.	Icteroides.
In envelopes, not sealed.....	+	+	—	—	—	—
In Petri dishes.....	+	—	—	—	—	—
In paper boxes, perforated ends.....	+	+	+	—	—	—

NOTE.—Throughout these experiments + means growth, and — no growth.

From this experiment it appears that the formaldehyd was strong enough in the six hours' exposure to kill all the non-spore-bearing organisms and some of the spores, but that it had no effect upon the mosquitoes, protected as they were by various layers of towel or cotton.

As the insects and the bacteria were not exposed under precisely similar conditions, the following experiment was made:

No. 2.

The conditions of this experiment were similar to those of No. 1, excepting the time of exposure was lengthened to twenty hours. The same room of 2,000 cubic feet capacity was used and 20 ounces (600 c.c.) of formalin (40 per cent) were sprayed upon 4 sheets.

This time the mosquitoes and the paper slips impregnated with the cultures of bacteria were exposed under exactly the same conditions. The insects were confined in little pill boxes with the gauze covers described above. The slips of paper impregnated with the cultures were placed in precisely similar boxes, the only difference being that the boxes were sterilized before receiving the slips. One series of boxes contained the mosquitoes, another subtilis spores, and the third pyocya-

neus. These 3 series were exposed side by side under the conditions as noted in the following table :

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyanus.
In pill boxes:				
In pocket of duck coat hung on wall, pocket toward wall..	3	1	—	—
Same as above, except pocket open and turned out.....	5	0	+	—
Under 2 layers of damp toweling.....	5	7	+	—
Under 2 layers of dry toweling ..	4	0	—	—
In pocket of overalls hung on wall, pocket closed and hung against the wall.....	1	4	+	—
Control; exposed to the gas without protection	4	0	—	—

It is perfectly plain from this experiment that the mosquitoes exposed freely to the action of formaldehyd are killed. They are, however, able to survive an exposure to less concentrated atmospheres of the gas. They lived in the pocket of a coat and overalls, and under a few layers of toweling, despite the fact that the gas penetrated to these places in sufficient strength to kill pyocyanus and sometimes dry subtilis spores, lying right alongside of the insects, and exposed to precisely similar conditions.

The conclusion is evident, therefore, that in rooms where the mosquitoes may have similar places to hide, this method is not available.

THE KUHN LAMP.

This lamp operates upon the principle of dehydrating methyl alcohol into formic aldehyd. The vapor of the alcohol is passed over incandescent platinum in a state of fine division upon perforated asbestos discs. In practical disinfection against bacteria, 20 ounces of wood alcohol are prescribed for each 1,000 cubic feet of air space, the exposure to be not less than six hours.

No. 3.

In the room of 2,000 cubic feet capacity described in experiment No. 1, 40 ounces of wood alcohol were consumed in a Kuhn lamp. The time of exposure was twenty-four hours. Temperature 81° to 87° F. The mosquitoes and the slips containing the cultures were in similar boxes, and exposed side by side to the same conditions, as follows :

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyanus.
In pill boxes:				
Under 1 layer damp toweling.....	1	2	+	—
Under 2 layers damp toweling.....	1	2	+	—
Under 4 layers damp toweling.....	4	0	+	—
Under 4 layers dry toweling	2	1	+	Lost.
Wrapped in dry towel, 4 layers.....	5	0	+	—
Wrapped in damp towel, 4 layers.....	4	0	+	+
In overalls pocket (damp), hung up, pocket toward wall...	0	4	+	—
In overall (dry), hung up, pocket turned out.....	0	4	+	—
In canvas mail sack, mouth tied.....	0	4	+	— ^a
Under pile of loose clothes.....	1	3	+	+
Control, in air-tight jar, dry.....	0	3	+	+
Control, in air-tight jar, with moisture.....	2	1	+	+

^a Contaminated.

The conditions of this test corresponded about to the requirements of bacterial disinfection, the time of exposure being longer than necessary to destroy surface infection.

From this test it is again plainly shown that the formaldehyd gas penetrates in sufficient strength to kill a non-spore-bearing organism (*pyocyaneus*) into pockets of overalls or through 1 layer of toweling, but not in sufficient strength to kill mosquitoes. There seems to be an analogy between these insects and the resistance shown by the spores.

The fact that some of the mosquitoes die when confined in a little pill box for twenty-four hours should not be taken as an indication that they were killed by the gas, for mosquitoes thus confined, even under favorable circumstances of moisture and temperature, often die. It is very common in our controls to find half or more of the insects dead within twenty-four hours; therefore, if 1 mosquito survives the exposure, we consider that the death of the others in the same box was not due to the effects of the gas.

No. 4.

This test was made in a small closet with 1 window, capacity, 250 cubic feet. About 17 ounces (500 c. c.) of wood alcohol were consumed in a Kuhn lamp. This is an excess of nearly 4 times the usual quantity required in disinfection. The room is of fairly tight construction and the door and window were closed without sealing or pasting. The temperature during the exposure was 82° to 86° F. The time of exposure, twenty hours.

The mosquitoes and the bacteria in this test were exposed in the pill boxes to the same conditions, as follows:

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyaneus.
In pill boxes:				
Under 2 layers of damp toweling.....	3	2	+	—
Under 2 layers of dry toweling	5	0	+	—
In pocket duck coat, hanging on wall, pocket turned to wall.....	6	0	+	—
In pocket duck coat, pocket stands open.....	4	0	—	—
In closed pocket of trousers, hung up.....	4	0	+	—
Controls, exposed without protection.....	3	0	+	—

It is evident that even excessive amounts of the gas, such as were employed in this test, are not sure insecticides if the mosquitoes are not exposed directly to the action of the gas. Note that 2 out of 5 mosquitoes survived under 2 layers of damp toweling. Despite the large amount of wood alcohol oxidized in the lamp, there was not enough formaldehyd gas generated to kill the subtilis spores exposed as controls.

THE TRENNER-LEE FORMALDEHYD DISINFECTOR.

This apparatus depends upon the principle of generating the formaldehyd gas from its watery solution, plus 1 per cent of glycerin, by distillation from a retort without pressure. For the purposes of disinfection against bacteria 12 ounces of formalin (40 per cent) are prescribed for each 1,000 cubic feet of air space.

No. 5.

This experiment was made in the same room, of 2,000 cubic feet capacity, as No. 1, and, as before, no calking or pasting was done. Twenty ounces of formalin (40 per cent), with the addition of 1 per cent of glycerin, were distilled in the Trenner-Lee apparatus. It required about forty-five minutes to evaporate this quantity. The room was kept closed six hours.

The cultures of bacteria were exposed as follows:

	Paper slips impregnated with—					
	Garden earth.	Subtilis spores.	Anthrax spores.	Staphylococcus.	Pyocyanus.	Icteroides.
In envelopes, not sealed.....	+	+	+	—	—	—
In Petri dishes, open.....	+	+	—	—	Lost.	—
In paper boxes, perforated ends.....	+	+	+	+	—	—

Mosquitoes were exposed as follows:

	Dead.	Alive.
In a battery jar containing crumpled paper and a crumpled towel, loosely filling about half the jar (the object was to afford a hiding place for the mosquitoes).....	0	12
In pill boxes—		
Covered with 1 layer toweling.....	0	5
Covered with 2 layers toweling.....	0	6
Covered with 4 layers toweling.....	0	4
Under 2 layers moist tissue paper.....	0	5
Wrapped in 4 layers damp toweling.....	0	8
Wrapped in 6 layers damp toweling.....	0	7
Rolled 4 times in a towel, ends not closed.....	2	6
Rolled 10 times in a towel, ends not closed.....	0	4

In this experiment the insects succeeded in hiding in the remote folds of the paper and towel in the battery jar, so that they escaped the effects of the gas, which was in sufficient strength to kill most of the non-spore-bearing organisms. Almost all the insects survived the other conditions of this test. Short exposures (six hours) to the ordinary percentages are, therefore, not enough to kill mosquitoes with as slight a protection as 1 layer of a crash towel.

No. 6.

This experiment was done under the same conditions as the preceding. Six hundred cubic centimeters, or about 20 ounces of formalin, were again used in the Trenner-Lee formaldehyd disinfecter. The exposure this time, however, was eighteen hours, and the mosquitoes and cultures were placed under precisely similar conditions, as follows :

	Mosquitoes.		Bacteria.					
	Dead.	Alive.	Garden earth.	Subtilis spores.	Anthrax spores.	Pyocyanus.	Staphylococcus.	Icteroides.
In battery jar with crumpled towel loosely filling half of it.....	2	1	+	+	+	—	—	—
In pill boxes—								
Under 1 layer of newspaper	2	0	+	—		—	—	—
In corner of newspaper, folded twice.....	2	0		+		—	—	—
Under 2 layers dry toweling....	2	0	+	+	+	—	—	—
Under 4 layers dry toweling ...	1	0	+	+	—	—	+	—
Wrapped tightly in a small towel	2	1		+		—	—	—
In open pocket of coat, hung up.....	2	0		—	+	—	—	—
In open pocket of duck coat, hung up	2	0	+	+	—	—	—	—
On the floor under several towels	1	1			+			

These results are better than No. 5, in which the exposure was only six hours, the other conditions being similar. However, one of the mosquitoes escaped destruction in the battery jar, one tightly wrapped in a towel, and one on the floor under several towels laid loosely in a heap over the boxes containing the insects. These conditions might be found in actual practice, especially in bedrooms containing heaps of clothing, bedding, or other fabrics where the mosquitoes, according to our observations, could readily find a hiding place where they might escape the action of the formaldehyd.

In this test the gas was in sufficient concentration to kill all the non-spore-bearing bacteria, excepting a staphylococcus under 4 layers of toweling. Some of the spores were also killed under conditions not requiring deep penetration.

No. 7.

In the third test with the Trenner-Lee formaldehyd disinfecter a small room containing 250 cubic feet was used. This room is the same as the one described under No. 4. The single window was simply closed without pasting the cracks. Five ounces of formalin with 1 per cent of glycerin

were distilled. The time of exposure was twenty hours. The mosquitoes and the cultures were exposed in pill boxes under similar conditions, as follows:

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyaneus.
In pill boxes—				
Controls, exposed to the gas without protection.....	5	0	+ <i>a</i>	—
Under 2 layers of dry toweling.....	4	0	— <i>b</i>	—
Wrapped tightly in dry towel, 6 layers.....	7	0	—	—
Under 2 layers of a damp towel.....	6	0	+	—
In pocket, duck coat, hung up, with pocket toward wall...	7	0	+	—
In pocket of overalls, folded once and laid on floor, pocket next to floor.....	4	2	+	—
In trousers pocket, deep, hung up.....	0	4	+	—
In corner of a folded newspaper, under 7 layers.....	2	6	+	—
On floor under towels, heaped in a pile.....	2	4	+	—
In canvas mail sack, mouth closed.....	3	3	+	—
In bottom of a battery jar, under 2 layers toweling.....	6	0	+	—

a Delayed.

b Contaminated.

The insects survived the twenty hours exposure in the pockets of overalls and trousers, under 7 layers of newspapers, under a heap of towels, and in a canvas mail bag. The insects under less severe conditions were all killed. The gas had practically no effect upon the dried spores, although it penetrated in sufficient quantity to kill all the pyocyaneus, despite the fact that some of the conditions required deeper penetration than is ordinarily the case in practical disinfection.

THE AUTOCLAVE.

The autoclave evolves the formaldehyd gas by heating formalin, mixed with a neutral salt, in a retort under a pressure of at least 45 pounds to the square inch.

The ordinary requirement with this form of apparatus in disinfecting against bacteria is to use 10 ounces of the formalin solution for each 1,000 cubic feet of air space with an exposure varying from two to twenty-four hours. The United States Quarantine Regulations require a twelve hours exposure and 400 c. c., or about 13 ounces per 1,000 cubic feet of space.

It is true that in this process not all the solution put in the retort is evaporated, and the amount remaining is noted under each experiment. However, there is very little formaldehyd in the residue, for in an apparatus evolving gas under pressure, especially if a neutral salt glycerin has been added to raise the boiling point, practically all the gas passes off with the first half of the liquid.

No. 8.

This experiment was done in the room of 2,000 cubic feet previously described. (See No. 1.) Twenty ounces (600 c. c.) of glyco-chloro-

formol was used. This is a mixture of formalin with 20 per cent of calcium chlorid and 10 per cent of glycerin. About 4 ounces of the solution remained in the retort after the operation. The time of exposure was twelve hours, making the conditions correspond about to the requirements as laid down in the United States Quarantine Regulations. The temperature of the room during this experiment was 72° to 87° F.

The mosquitoes and the cultures were exposed in the pill boxes, placed side by side, so that the conditions were as nearly alike as possible.

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyanus.
In pill boxes—				
Under 1 layer damp towel.....	2	2	+	—
Under 2 layers damp towel.....	3	1	+	—
Under 4 layers damp towel.....	0	2	+	—
Under 4 layers dry towel.....	2	5	+	—
Wrapped in 4 layers dry towel.....	0	1	+	—
Wrapped in 4 layers damp towel.....	0	3	+	—
In damp pocket of overalls, hung up.....	0	4	+	—
In dry pocket of overalls, hung up.....	10	5	+	—
In mail bag, mouth tied.....	0	8	+	— ^a
Under pile of towels, loosely heaped.....	1	4	+	—
On wall behind a duck coat.....	3	3	Lost.	—
Control, in air-tight jar, with moisture.....	2	2	+	+

^a Contaminated.

From this test it is plain that the requirements of the United States Quarantine Regulations, which are sufficient to destroy non-spore-bearing bacteria in the pockets of clothing or under 4 layers of towels, are impotent to destroy mosquitoes similarly protected. Some of the insects survived in each one of the boxes. Even in the control box 2 of them were dead. It is, therefore, plain that the occasional deaths in all the other cases can not be attributed to the effects of the gas.

The analogy between the resistance of the spores and the resistance of the mosquitoes is strikingly shown in this test.

No. 9.

The second experiment with the autoclave was done in the same room as the above, but under different conditions. The exposure was short and the percentage of formaldehyd very high. Two thousand cubic centimeters, or about 65 ounces, of the glyco-chloro-formol solution was used. This is 3 times the amount required for ordinary disinfection. After a pressure of 60 pounds to the square inch was obtained, the gas was allowed to enter the room. The operation was continued at this pressure for fifteen minutes. One-half of the liquid remained in the autoclave at the close of the operation.

The total time of exposure was two hours and fifteen minutes, counted from the time the gas was first allowed to enter the room. The temperature of the room during the test was 81° to 89° F.

The bacteria were exposed as follows :

	Garden earth.	Subtilis spores.	Anthrax spores.	Staphy- lococcus.	Pyocya- neus.	Icter- oides.
In envelopes, not sealed	+	—	—	—	—	—
In Petri dishes, open.....	+	—	—	—	—	—
In paper boxes.....	+	+	+	—	—	—

The mosquitoes were exposed as follows :

	Dead.	Alive.
In a battery jar—		
1. Covered with mosquito netting only.....	15	0
2. One-third full of crumpled toweling and tissue paper loosely tucked in to afford a hiding place.....	1	11
3. Two-thirds full of paper and toweling to afford a hiding place...	0	12
In pill boxes—		
1. Between sheets of a folded newspaper.....	0	2
2. Under 1 sheet of newspaper.....	8	0
3. Under 1 layer of dry toweling.....	6	0
4. Under 2 layers of dry toweling.....	1	2
5. Wrapped in 1 layer of damp toweling.....	7	0
6. Wrapped in 2 layers of damp toweling.....	6	1
7. Wrapped tightly in small damp towel.....	0	4
8. Wrapped twice in a damp towel and that covered with a dry towel	0	4
9. Stuck to wall with a damp towel hung directly over the box....	8	1
10. Same with a dry towel hung over it, 1 inch from box.....	14	0
11. Same with 2 dry towels hung over the box.....	8	0
12. In outside overcoat pocket, 8 inches deep.....	0	7
13. In inside pocket of overcoat.. ..	0	5
14. On floor, under 2 crumpled towels, piled in a heap.....	0	4
15. On floor under a pile of towels.....	0	2

The mosquitoes and the cultures were not exposed under precisely similar conditions, so that an exact comparison can not be drawn between the insecticidal and germicidal power of the gas in this test.

Here it is again evident from the tests made with the battery jars that the mosquitoes have the ability to protect themselves by seeking hiding places in the folds of the towel and tissue paper.

In this test the high percentage of the gas had sufficient germicidal power to kill all the non-spore-bearing organisms and many of the spores during the short exposure.

No. 10.

This test was made with the autoclave in the same room of 2,000 cubic feet capacity described above. The gas was allowed to pass into the room for fifteen minutes under a pressure of between 45 and 50 pounds to the square inch. As before, 2,000 c. c. (about 65 ounces) of the glycochloro-formol solution were used and 1,200 c. c. passed over, leaving a residue of 800 c. c. in the retort.

The time of exposure was nineteen hours, the temperature of the room being 75° to 85° F.

The mosquitoes and cultures were exposed in the small pill boxes under exactly similar conditions as follows:

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyanus.
In pill boxes:				
Exposed to the gas without protection.....	5	0	+ <i>a</i>	—
Under 2 layers of dry toweling.....	5	0	+	—
Wrapped tightly in a dry towel, 8 layers.....	6	0	+ <i>a</i>	—
Under 2 layers of damp toweling.....	8	0	— <i>b</i>	—
Wrapped tightly in a damp towel, 8 layers.....	2	3	+	—
In pocket of a linen coat, hung on wall.....	4	0	+	—
In pocket of duck trousers, folded and laid on floor with pocket down.....	2	3	+	—
In pocket of trousers, hung up, pocket to wall.....	1	3	+	—
In corner of a folded newspaper, under 7 layers.....	4	0	+	—
Under 1 yard of toweling, heaped loosely in a pile.....	0	5	+ <i>a</i>	—
In canvas mail sack, mouth tied.....	6	0	+	—
In bottom of a battery jar under 2 layers of toweling.....	7	0	+	—

a Delayed.

b Contaminated.

Here an excess of about 3 times the usual amount of formalin solution was used, and the time of exposure was nineteen hours. Under these severe conditions, most of the mosquitoes succumbed. The only survivors were found under exceptionally severe conditions, such as under a heap of towels, or through 8 layers of moist towels, or in the pocket of trousers, folded and laid on the floor with the pocket down. The gas penetrated in sufficient strength to destroy all the non-spore-bearing bacteria exposed under precisely the same conditions in boxes alongside those containing the mosquitoes. None of the dry spores were killed.

It is evident from these tests with the autoclave that the time of exposure is a very important factor in obtaining penetration of the gas to the insects protected in fabrics, etc.

Large percentages of gas for a short time will not penetrate; neither do the conditions prescribed by the United States quarantine regulations appear to be effective in penetrating to insects deeply hidden, while the long exposures to a large percentage of gas gives but moderately satisfactory results, as far as penetration is concerned. The mosquitoes directly exposed to any of these conditions are all killed in a very short time.

While it is by no means absolute, the similarity between the resistance of spores and mosquitoes to formaldehyd disinfection is very striking. In other words, to be sure of killing mosquitoes in an ordinary room, containing fabrics where the insects may hide, we must use a concentration of the gas and a length of exposure sufficient to kill a spore-bearing organism.

SULPHUR DIOXID.

In order to compare the merits of formaldehyd with sulphur dioxid gas in disinfection against mosquitoes, similar experiments were made by burning sulphur and with the liquid sulphur dioxid gas.

The power of sulphur dioxid to destroy all forms of animal life is well known. On account of its destructive action upon fabrics and metals, this agent is of little practical use in the disinfection of dwelling houses, cabins of ships, and similar places. This destructive action is due to the moisture which combines with the sulphur dioxid to form sulphurous acid, which is the real disinfecting agent. Dry sulphur dioxid has practically no effect upon bacteria. Our work has shown that very small atmospheres of the dry gas will quickly destroy mosquitoes, and we therefore believe that the destruction of these insects may be accomplished in dwelling houses with little danger of injuring fabrics or tarnishing metals. Sulphur dioxid is so far superior to formaldehyd as an insecticide that even this risk should not outweigh the certainty of its action. The gas may now be obtained in its liquefied form, either in tin cans, in syphons, or in iron cylinders, affording very convenient methods of quickly introducing a given amount of the dry gas into an inclosure.

No. 11.

Two pounds of sulphur were burned in a small room with a capacity of exactly 500 cubic feet. This gives nearly the prescribed 4 per cent of gas. The room was specially built for experiments with gaseous disinfectants and is made very nearly air-tight. The time of exposure was four hours.

The sulphur was in the form of "flowers." It was burned in a small pot and the fire started with alcohol.

Mosquitoes were exposed in the small pill boxes with gauze lids, as follows :

In pill boxes—	Dead.	Alive.
Under 1 layer toweling.....	4	0
Under 2 layers toweling.....	7	0
Under 4 layers toweling.....	4	0
Wrapped twice in towel.....	3	0
Covered with 1 sheet newspaper.....	5	0
In corner folded newspaper.....	5	0
In inside pocket of overcoat.....	3	0
On wall back of coat.....	4	0
On wall back of 3 towels.....	4	0
On floor under several loose towels.....	4	0
In battery jar one-third full crumpled toweling.....	12	0

No bacteria were exposed in this test. The result showed that the prescribed amount of sulphur will kill the insects in a much shorter time than is required to destroy bacterial infection, and that the sulphur dioxid possesses considerable penetrating power in this short time.

No. 12.

In the same room of 500 cubic feet capacity 330 grams of sulphur (about one-third the prescribed amount) were burned, and the room was left closed twenty-four hours. Mosquitoes were exposed and with some of them subtilis spores, fresh garden earth, and pyocyaneus germs were exposed in boxes under similar conditions, as follows:

	Mosquitoes.		Bacteria.		
	Dead.	Alive.	Garden earth.	Subtilis spores.	Pyocyanus.
In pill boxes:					
On floor, no protection.....			+		
Under heavy carpet.....				+	
On shelf, no protection.....			+		
On rafter near ceiling, no protection.....			+		
In corner, folded newspaper.....	6	0		+	—
Under 4 thicknesses toweling.....	6	0		+	—
In mail sack half full of crumpled paper.....	7	0			—
Under 8 layers dry toweling.....	5	0			
Under 4 layers damp toweling.....	5	0			—
Under 8 layers damp toweling.....	5	0			—
In pocket duck trousers, rolled up.....	6	0			
Rolled 4 times in damp towel and put in coat pocket.....	6	0			
On floor under empty wooden box.....	5	0			

This small amount of the gas killed all mosquitoes and non-spore-bearing organisms even under conditions requiring deep penetration.

No. 13.

These results with sulphur dioxid were so good that another test was made to see whether a very small amount of sulphur with a short exposure would be sufficient to kill mosquitoes and leave the non-spore-bearing organisms unharmed.

To this end about 6 ounces (191 grams) of sulphur were burned by the pot method in a room of just 500 cubic feet capacity. The room was aired one hour after the sulphur was lighted, so that the total exposure to the gas was one hour.

The mosquitoes and the cultures were exposed in the pill boxes with gauze lids, under similar conditions, as follows:

	Mosquitoes.		Cultures.
	Dead.	Alive.	Pyocyanus.
In pill boxes:			
One shelf without protection.....	4	0	+
On high rafter, no protection.....	4	0	+
Under 1 layer damp toweling.....	4	0	+
Under 2 layers damp toweling.....	4	0	+
Under 4 layers damp toweling.....	4	0	+
Wrapped in damp towel, 4 layers.....	3	0	+
Wrapped in damp towel, 8 layers.....	0	4	+
Wrapped in dry towel, 4 layers.....	4	0	+
On floor under a dirty sheet.....	2	3	+
On floor under a wooden box.....	0	4	+

It is plain that very weak percentages of sulphur dioxid are sufficient to kill mosquitoes in a short time, even though protected by 4 layers of

toweling. None of the cultures were killed. Sulphur dioxid has always been regarded as merely a surface disinfectant against bacterial infection. The results of all who have tested the gas are unanimous in concluding that it has very feeble powers of penetration. Its effect upon mosquitoes is in striking contrast to its bactericidal action in this regard.

EXPERIMENTS WITH LIQUID SULPHUR DIOXID.

No. 14.

One tin can containing 20 ounces of liquid sulphur dioxid gas was opened in the room containing 500 cubic feet. The temperature in the room was 29.3° to 30° C. Exposure eighteen hours. One of these 20-ounce cans is supposed to suffice for 1,728 cubic feet, so that the amount used was about three and a half times that required for bacterial disinfection.

The mosquitoes and the cultures were exposed in the pill boxes with gauze lids, under similar conditions, as follows :

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyanus.
In pill boxes:				
On floor, under carpet.....	6	0	+	—
In canvas mail sack, folded.....	8	0	+	—
In pocket, duck coat, hung up.....	7	0	+	—
In pocket, cloth trousers, hung up.....	7	0	+	—
Under 2 layers, dry toweling.....	6	0	+	—
Under 2 layers, damp toweling.....	5	0	+	—
Wrapped tightly in dry towel, 7 layers.....	5	0	+	—
Wrapped tightly in damp towel, 7 layers.....	5	0	+	—
In coat pocket, hung up, flap closed.....	4	0	+	—
In bottom 12-inch beaker, covered with 2 layers toweling...	6	0	+	—
In corner folded newspaper, under 7 layers.....	7	0		

The moisture of the atmosphere during this test was determined by drawing the atmosphere over calcium chlorid. The gain in weight showed the presence of 0.2692 grams of moisture per liter.

No. 15.

A second test with the liquid sulphur dioxid was made. The conditions were precisely similar to those of the preceding experiment, No. 14, excepting that moisture in the form of steam was injected through the keyhole after the gas was liberated. The temperature of the room during the exposure was 29.3° to 30° C. The atmosphere was saturated with moisture.

The mosquitoes and the cultures were exposed in the pill boxes with gauze lids, under similar conditions, as follows:

	Mosquitoes.		Bacteria.	
	Dead.	Alive.	Subtilis spores.	Pyocyaneus.
In pill boxes:				
On floor, under carpet.....	7	0	+	—
In canvas mail sack, folded.....	5	0	+	—
In pocket duck coat, hung up.....	5	0	+	—
In pocket cloth trousers, hung up.....	5	0	+	—
Under 2 layers dry toweling.....	9	0	Lost.	—
Under 2 layers damp toweling.....	7	0	+	—
Wrapped tightly in dry towel, 7 layers.....	6	0	+	—
Wrapped tightly in moist towel, 7 layers.....	7	0	+	—
In coat pocket, hung up, flap closed.....	6	0	+	—
In bottom of 12-inch beaker covered with 2 layers of towel.....	7	0	+	—
In corner of folded newspaper, under 7 layers.....	8	0	+	—

The results were the same as No. 14, viz, death of all mosquitoes, even when well protected by dry or moist fabrics; the death of fresh moist cultures of pyocyaneus, and the failure to affect subtilis spores similarly protected.

No. 16.

A series of experiments was made to determine whether chemically dry sulphur dioxid has insecticidal properties. It is well known that the anhydrous gas has practically no effect upon bacteria. As the dry gas is not destructive to fabrics and metals, it is of considerable practical importance to know whether it will kill mosquitoes.

To this end the liquid sulphur dioxid was liberated in a bell jar, but first passed through 2 drying columns containing pumice stone saturated with sulphuric acid. The moisture contained in the air of the bell jar was eliminated in 2 ways, (1) by drawing air through the drying columns into the bell jar, or (2) by introducing calcium chlorid into the bell jar.

It was found, in all these tests, that the mosquitoes were killed, practically, instantly by the dry gas.

Contrary to formaldehyd, which requires an exposure and strength of gas sufficient to destroy spores in order to entirely rid a room of mosquitoes, sulphur dioxid will kill these insects even when the quantity of the disinfectant and the time of exposure are reduced so that non-spore-bearing bacteria are unharmed.

Sulphur dioxid was for a long time used as a disinfecting agent against yellow fever, and experience found it to be trustworthy. But later it was disparaged because laboratory tests showed that it lacked the power of killing spores and has little penetrating power through fabrics. But now that we know it is the mosquito which carries the infection, the usefulness of this agent is revived.

SUMMARY AND CONCLUSIONS.

FORMALDEHYD GAS is a feeble insecticide. Mosquitoes may live in a very weak atmosphere of the gas overnight. It will kill them, however, if it is brought in direct contact in the strength and time prescribed for bacterial disinfection. For this purpose any of the accepted methods for evolving the gas is applicable, but the methods which liberate a large volume in a short time are more certain than the slower ones.

Direct contact between the insects and the gas is much more difficult to obtain in ordinary room disinfection against mosquitoes than against germs, because the sense of self-protection helps the former to escape from the effects of the irritating gas. They hide in the folds of towels, bedding, clothing, hangings, fabrics, and out-of-the-way places where the formaldehyd gas does not penetrate in sufficient strength to kill them. The gas is polymerized and deposited as paraform in the meshes of fabrics, which prevents its penetration, and large quantities are lost by being absorbed by the organic matter of fabrics, especially woollens. In our tests, whenever the insects were given favorable hiding places, such as in crumpled paper or in toweling, they quickly took advantage of the best place for themselves and thus escaped destruction.

There is a striking analogy between the strength of the gas and the time of exposure necessary to penetrate the fabrics in order to kill mosquitoes, and the strength and time necessary to penetrate in order to kill the spores of bacteria.

Mosquitoes have a lively instinct in finding cracks or chinks where fresh air may be entering the room, or where the gas is so diluted that they escape destruction. They are able to escape through incredibly small openings. Some of the smaller varieties, such as the *stegomyia fasciata* can get through a wire screen having 12 meshes to the inch. Therefore, formaldehyd gas can not be trusted to kill all the mosquitoes in a room which can not be tightly sealed.

It is concluded, that to succeed in killing all the mosquitoes in a closed space with formaldehyd gas, the following definite requirements are essential: A very large volume of the gas must be liberated quickly, so that it may diffuse to all portions of the space in sufficient concentration. The room must have all the cracks and chinks where the insects may breathe the fresh air carefully sealed by pasting strips of paper over them. The room must not contain heavy folds of drapery, clothing, bedding, or fabrics in heaps, or so disposed that the insects may hide away from the full effects of the gas.

SULPHUR DIOXID is unexcelled as an insectide. Very dilute atmospheres of the gas will quickly kill mosquitoes. It is quite as efficacious for this purpose when dry as when moist, whereas the dry gas has practically no power against bacteria. Contrary to formaldehyd it has sur-

prising powers of penetrating through clothing and fabrics, killing the mosquitoes, even when hidden under 4 layers of toweling, in one hour's time—and with very dilute proportions.

This substance, which has so long been disparaged as a disinfectant because it fails to kill spores, must now be considered as holding the first rank in disinfection against yellow fever, malaria, filariasis, and other insect-borne diseases.



BULLETIN No. 7 OF THE HYGIENIC LABORATORY,

Treasury Department, U. S. Marine-Hospital Service,

WALTER WYMAN, *Surgeon-General.*

MAY, 1902.

LABORATORY TECHNIQUE.

RING TEST FOR INDOL,

S. B. GRUBBS AND EDWARD FRANCIS.

COLLODIUM SACS,

S. B. GRUBBS AND EDWARD FRANCIS.

MICROPHOTOGRAPHY WITH SIMPLE APPARATUS,

H. B. PARKER.

M. J. ROSENAU,

Passed Assistant Surgeon, Director Hygienic Laboratory,

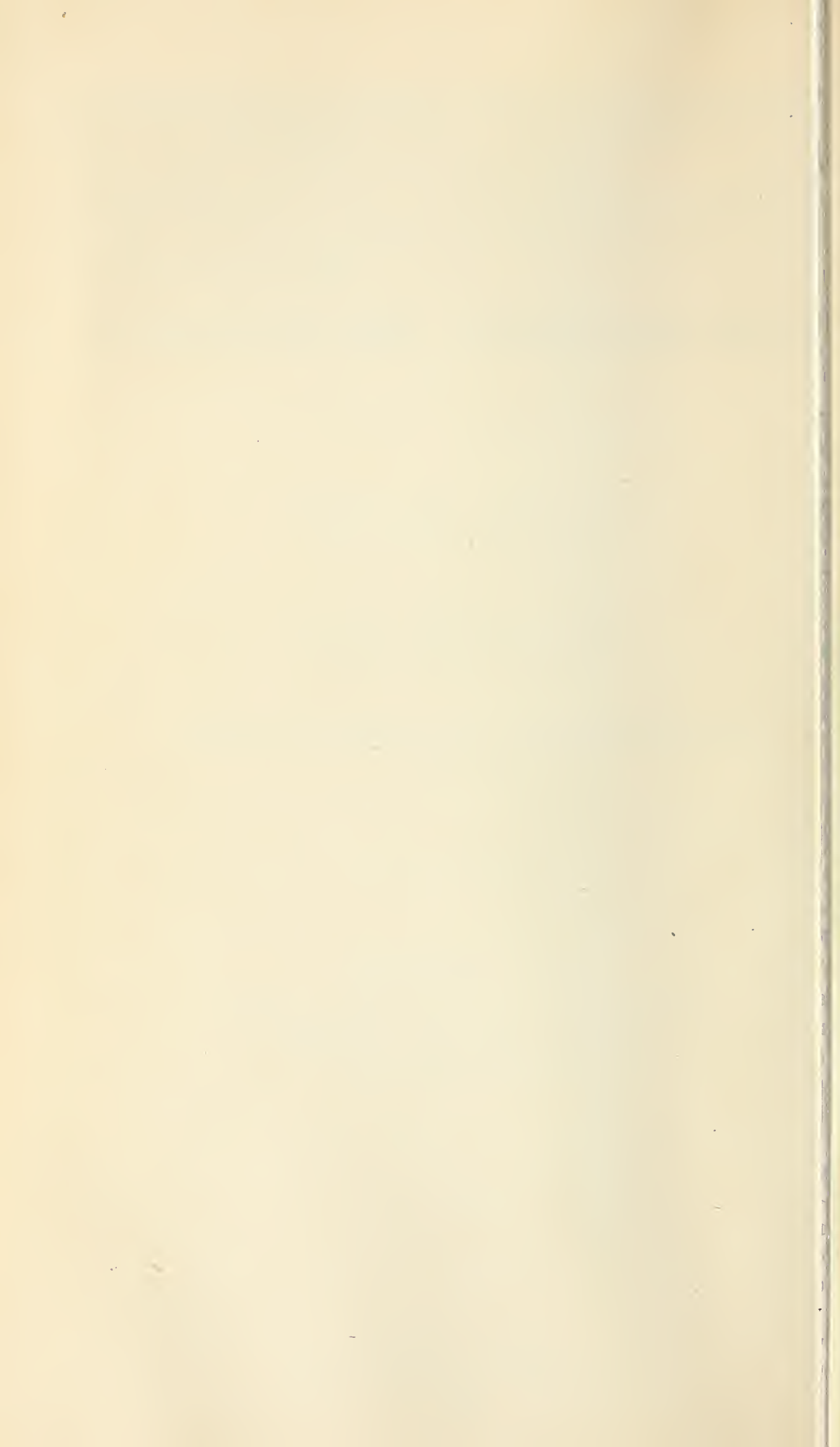
U. S. MARINE-HOSPITAL SERVICE,

WASHINGTON, D. C.

WASHINGTON:

GOVERNMENT PRINTING OFFICE.

1902.



RING TEST FOR INDOL.

(BY S. B. GRUBBS, PASSED ASSISTANT SURGEON, AND EDWARD FRANCIS, ASSISTANT SURGEON,
UNITED STATES MARINE-HOSPITAL SERVICE.)

The method of applying the indol test, as ordinarily used in laboratories and described in our text-books, is certainly crude, and consequently the statement that an organism does or does not produce indol leaves us in most cases very uncertain as to just what is meant.

As we know, the line between the indol producers and non-producers is not distinct, and several organisms "not producing indol" do give the reaction if grown long enough in suitable media. Thus, for example, we can get a faint but distinct reaction from cultures of *Bacillus pestis* and *Bacillus typhosus*, although these organisms under ordinary conditions are not considered indol producers.

So we must draw the line arbitrarily, and in this laboratory we take cultures grown twenty-four hours at 37° C. in media containing 1 per cent of peptone and use the ring or contact test, allowing it to remain in contact one hour.

We have applied the ring test in determining the presence of indol on account of its simplicity, its clearness, and its delicacy. When the acid and solution of nitrites were mixed, we found the color reaction was often unsatisfactory, the natural yellow tint masking the pink, or the color appearing on first contact with the nitrite solution would disappear when the tube was shaken.

The following technique will always give a clear-cut reaction if indol is present, or if not, there is no difficulty in deciding in the negative:

To 7 c.c. of the twenty-four-hour culture add 8 to 10 drops of concentrated sulphuric acid and shake; then let 3 or 4 c.c. of a 1-1000 sodium-nitrite solution run carefully down the side of the tube so as to form a sharp line of contact between the heavier culture and acid and the lighter nitrite solution, when the pink color will contrast sharply with the portions above and below.

The adoption of this technique, with the time limits mentioned, will make the indol reaction very clear in its results and will increase its value as a means of recognizing species.



COLLODIUM SACS.

(BY S. B. GRUBBS, PASSED ASSISTANT SURGEON, AND EDWARD FRANCIS, ASSISTANT SURGEON,
UNITED STATES MARINE-HOSPITAL SERVICE.)

Since the publication of the work of Metchnikoff, Roux, and Salimbeni¹ on the toxins of cholera, the growing of cultures in collodium sacs placed in the peritoneal cavities of animals has become a common procedure not only at the Pasteur Institute, but in all bacteriological laboratories when it is desired that the culture in its growth shall be raised in virulence through the action of the nutrient body fluids and yet be protected from the influence of the body cells and phagocytes.

As usually made, the sac is molded over the end of a glass rod by dipping it several times into the collodium solution, after which it is peeled off (Pasteur Institute).

Novy² slips inside the sac a glass tube which is perforated and constricted, and he fixes the free border of the sac to the glass tube at a point just below the constriction by means of heat, pressure, a silk ligature, and additional collodium.

McCrae,³ following Prudden, employs a glass tube, to the heated end of which he attaches a gelatin capsule which acts as a temporary frame over which the collodium is molded. The capsule is then removed by dissolving it in hot water.

In the techniques just referred to there are several delicate manipulations which greatly hazard the chances of getting a perfect sac:

1. There is the danger of tearing and destroying the sac in peeling it off the rod.

2. There is considerable difficulty in attaching the sac securely to the glass frame; and,

3. If no frame is used, the sac has no support, may become shriveled, and is liable to tear on removal from the animal's abdominal cavity if bound around by adhesions.

¹Toxine et antitoxine cholérique Ann. de l'Institut Pasteur 1896. X.

²Laboratory work in bacteriology.

³Journal of Experimental Medicine, vol. 5.

In endeavoring to overcome these difficulties we have hit upon the following simple technique and have used it repeatedly in the Hygienic Laboratory for several months:

The sac is molded in the very beginning over the perforated glass frame, from which it is not later removed.

The steps are as follows:

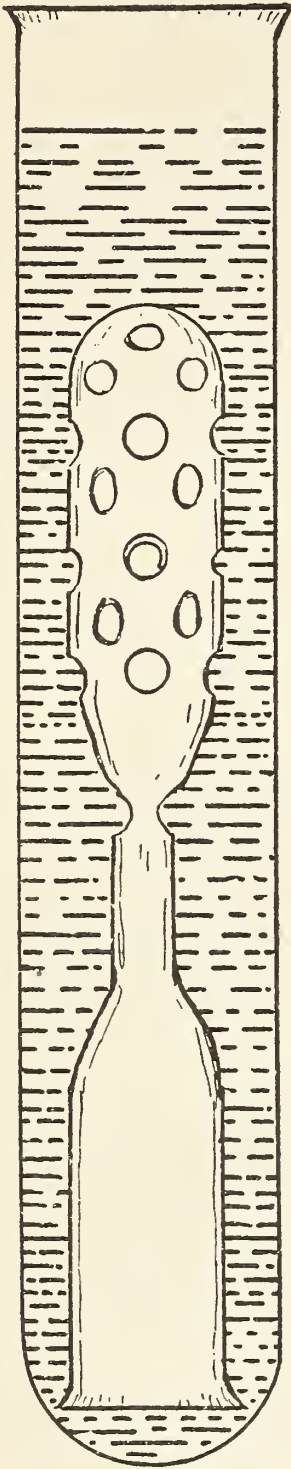
A glass frame is first made according to the method of Novy. Eight to twelve openings, each having a diameter of about $\frac{1}{8}$ inch, are blown as closely as possible in the lower part of a small test tube, the size and number of the holes depending on the amount of osmosing surface desired.

The tube is then partially constricted in the flame at a point just above the openings. The constriction is of such size as to admit a fine pipette for inoculation. Some pure gelatin, to which is added a little water, is liquefied by heat, and with a glass rod a drop of the hot gelatin is applied to each individual opening in the glass frame, which, when it solidifies, forms a thin film which temporarily closes each perforation.

The tube is now dipped two or three times into the collodium solution to a point well above the openings but below the constriction. In order to remove the gelatin film from the holes, the tube is filled with water and it is inverted in a larger tube or beaker of water the temperature of which is raised until the gelatin melts and runs down the inside of the tube through the constricted part and out into the water (see cut).

We then have the collodium firmly adherent to the glass frame, covering its openings, well supported and ready to stand, without tearing or leaking, the manipulations of sterilization, filling, inoculation, sealing, implantation and removal from the abdominal cavity.

The ease with which this technique can be carried out will, we think, appeal to those who have had experience with other methods.¹



The glass frame inclosed in a test tube of hot water to remove the gelatin from the perforations.

¹ The glass frame may be further improved by directing the gas blast flame against the tube on opposite sides just below the constriction until the glass becomes softened and indented. These two flattened surfaces afford a good place for grasping the frame with a pair of forceps during the sealing off and implantation. This overcomes the slipping and turning which occur on attempting to hold firmly a perfectly round tube.

MICROPHOTOGRAPHY WITH SIMPLE APPARATUS.

(BY H. B. PARKER, ASSISTANT SURGEON, UNITED STATES MARINE-HOSPITAL SERVICE.)

The art or mechanics of photography has in the last few years become very useful in pathological, histological, and bacteriological work. Almost every laboratory is supplied with an approved apparatus, while the independent workers convert their photographic apparatus, either kodak or camera, into some form of apparatus to meet their particular desires or needs. As a reward for their efforts they meet with varying degrees of success, so much so that in modern medical literature it is impossible in many instances to determine from the results whether the writer had in his possession an approved model or a simple makeshift of his own.

With low diameters almost any combination of microscope, camera, and illuminant will produce a fair picture in the hands of an amateur familiar with outdoor photographic processes. To obtain success with the higher diameters special apparatus is required, and these, I regret to say, are so expensive and difficult of manipulation that their use is prohibited in any but the more luxuriously equipped laboratories. The average size of the picture desired in bacteriological work is 2 inches; for pathological work somewhat larger; the average size of the plate in the modern microphotographic outfit is $6\frac{1}{2}$ by $8\frac{1}{2}$ inches to 8 by 10 inches with a bellows extension that will average 5 feet. This long extension was for a time an advantage, permitting the use of low-power objectives, amplification being secured by distance, but with the advent and perfection of apochromatic objectives, compensation and projection oculars, and instruments of greater precision in sectioning pathological and histological processes, the use of the long bellows is practically limited to achromatic and specially devised photographic objectives. A disadvantage probably more prominent than the preceding is the necessity of an arc light with its elaborate system of condensers and absorbing cells as the illuminant, a system too complex for the small and moderate size laboratory.

To overcome some of the disadvantages mentioned we have constructed an apparatus on the usual principle of the horizontal instrument but have reduced the size of the bellows extension to 32 inches and the size of the plate to 4 by 5 inches. We used an ordinary lantern-slide camera, which gave the results shown in the illustrations.

DESCRIPTION OF THE APPARATUS.

Success in microphotographic work depends largely upon the illuminant, of which there are six for consideration: Sunlight, the electric arc light, the oxyhydrogen light, illuminating gas, coal oil, and acetylene. Sunlight gives excellent photographs but is excluded on account of the expensive apparatus and special requirements for good work. The electric arc gives excellent results and is necessary when magnifications above 1,500 diameters are desired. The oxyhydrogen light, illuminating gas, and coal oil are too feeble for any but low diameters. Acetylene, because of its brilliancy, high actinic properties, low heat reflection, portability, low cost, and adaptability to other laboratory work, is the illuminant par excellence for diameters up to and including 1,500, for low-power microscopic projection, and lantern-slide projection.

The illuminating apparatus in use at this laboratory consists of a generator large enough to furnish gas for a quadruple burner, two condensers, of 4-inch focus, and a light filter and an absorbing cell holding a layer of water $\frac{1}{2}$ inch thick. The latter is sufficiently large to remove all heat rays as well as serving to hold water tinged with a color complementary to the stain employed on the specimen to be photographed.

The bellows as described above is one of the numerous models for lantern-slide work, either $3\frac{1}{4}$ by $4\frac{1}{4}$ or 4 by 5 inches.

The whole apparatus is mounted upon a firm base.

FOCUSING.

One of the most difficult features in connection with microphotographic work is focusing the object on the ground-glass screen. We have discarded the various focusing rods used for this purpose and now depend upon a braided twine, the center going around the fine adjustment and thence to the back of the camera, where it is kept taut by small weights. This has the advantage that when the focus is accurately found the ends can be released, removing the strain from the fine adjustment and consequently all interference with the focus. Rods clamped to the fine adjustment are apt to change the focus when released and are acted upon by vibrations of the room.

A magnifying glass of low magnification can be used as an aid in focusing with high diameters, but as a rule the focus of the entire field can be much more accurately gauged with the eye alone than with the aid of lenses.

EXPOSURE.

The length of exposure will depend upon the intensity of the image upon the screen and upon the rapidity of the plate. A

rapid exposure with an excess of light does not give the details which are obtained with less light and a longer exposure. In a general way then it may be said that the intensity of light should be subdued by reducing the diaphragm in the Abbe condenser until the exposure will approximately require about five seconds.

Care should be taken to see that no vibrations are produced during the exposure. This is best accomplished by selecting a time when the streets are free from passing vehicles, and all in the room should remain quiet. An ordinary pneumatic photographic shutter in the front board serves to reduce vibrations to a minimum and is far superior to the regular shutter supplied with these cameras.

CHOICE OF PLATES

Only plates of double coating should be selected for this work, preferably one of the medium or slow grades.

They are distinguished from the ordinary plates by their isochromatic or orthochromatic qualities, giving correct color values with double-stained preparations—a property that can be increased by using a color screen complementary to the object to be photographed.

As a rule with these plates, no color screen is required with fuchsin, safranin, hæmatoxylin, or carmine.

Without reference to the complementary color, a color screen made with chromic acid or sulphate of copper, or both, combined in weak solution will answer for any color or combination of colors in general laboratory use.

DEVELOPMENT.

A good developer has the property of producing both detail and density. A single developer does not possess these qualities perfectly, so a combination is used.

Metol and hydrochinone about represent the extremes, but when combined in proper proportions give the best results in microphotographic work, as well as in general photography. The formula most used is the following:

R	
Metol	2.00
Hydrochinone	2.00
Sodium sulphite	20.00
Sodium carbonate	10.00
Potassii bromidi	0.25
Distilled water	500.00

M.

The metol and hydrochinone should be dissolved in the water before adding the sulphite. This preparation keeps indefinitely, and may be used until exhausted. Whatever the exposure, the development should be carried out until the plate is completely developed; errors in over exposure or under exposure may then be corrected by reduction or intensification, either process with full development producing a better negative than one poorly developed.

Formulæ containing a mercury salt, for intensification, or containing potassium ferricyanide, for reducing, usually accompany all makes of plates.

COST OF TEMPORARY AND PERMANENT APPARATUS.

The advantage of this apparatus is the low primary cost. A lantern-slide camera at a cost of \$10 makes an excellent bellows when firmly mounted on a frame. The generator, burner, two condensers and light filter would cost about \$25, making a total cost of about \$35.

A complete outfit with the necessary mechanical arrangements for centering the light, varying the focus of the condensers, and time shutter should be supplied for about \$60.

Where a laboratory is supplied with electricity, that illuminant may be used by substituting a hand-feed electric arc and a large absorbing cell at an increase in cost of \$5 to \$10.

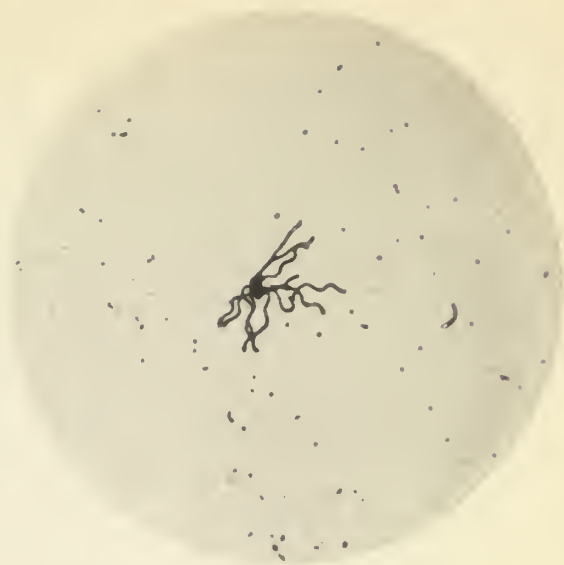
With this apparatus and the well-known apochromatic objectives and projective oculars of Zeiss, it is possible to take a photograph of 1,000 diameters in two seconds, or about as rapidly as an exposure can be accurately made. For the low diameter the light is stopped down until the desired illumination is obtained. Our experience is that an exposure of five seconds for all magnifications gives greater detail than a more rapid exposure. In this time vibrations do not seriously interfere with sharpness of outline. A printing-out silver paper gives the best tone to a photograph when half-tone reproduction is desired. The finished print should be a shade darker, as density is always lost in the process of reproduction.

EXPLANATION OF PLATE No. 1.

1. Tetanus bacilli, with spores.
2. Typhoid bacillus, with flagella.
3. Plasmodium malariae, in human blood, crescentic forms.
4. Staphylococci in pus.
5. Normal kidney, mouse.
6. Vaccine bodies in cornea of rabbit.



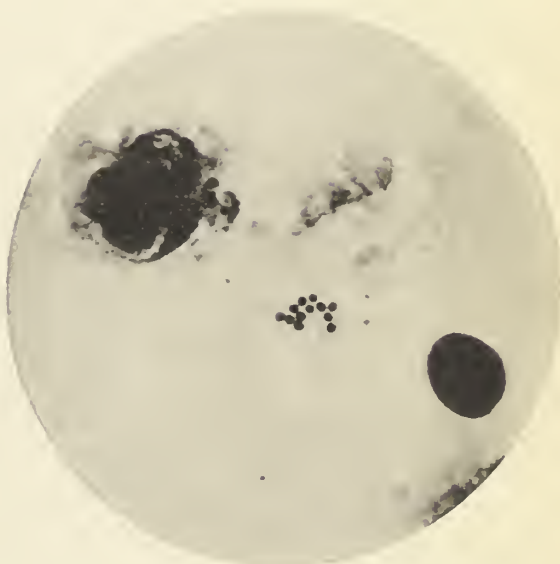
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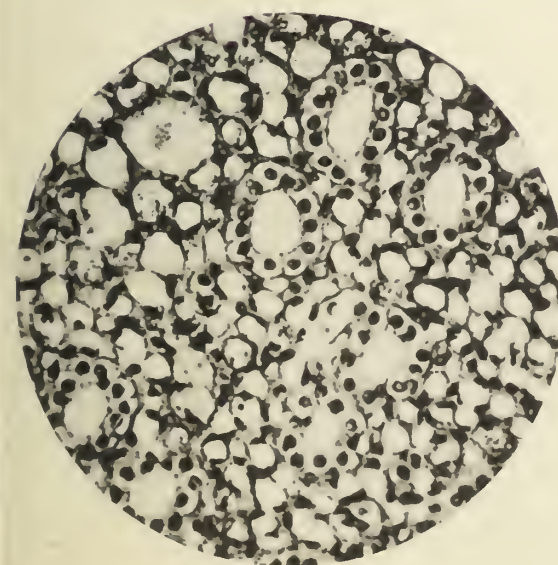
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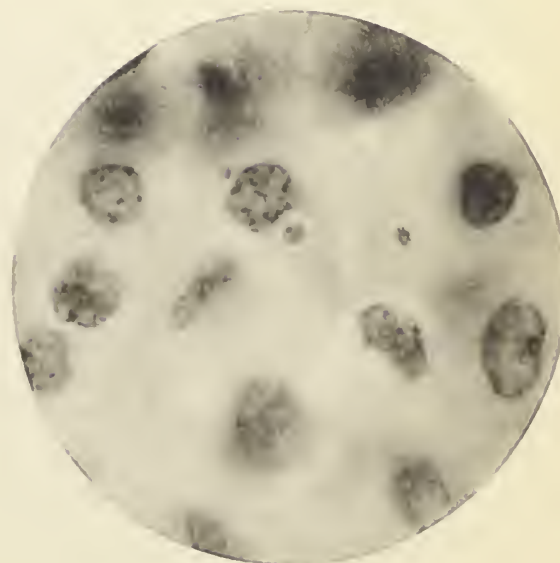
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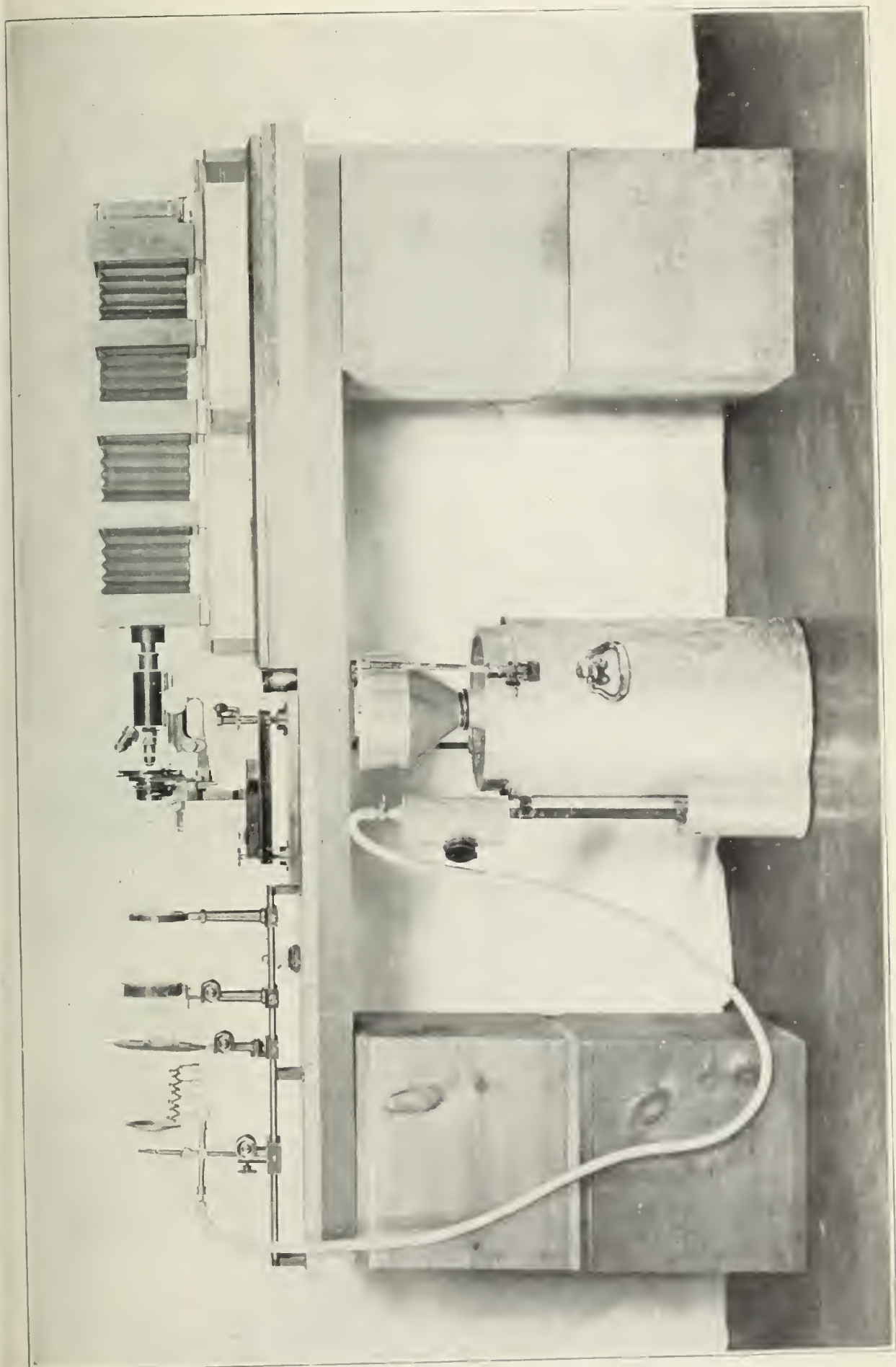


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6





TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.
WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 8.

AUGUST, 1902.

LABORATORY COURSE

IN

PATHOLOGY AND BACTERIOLOGY

BY

M. J. ROSENAU,
DIRECTOR OF HYGIENIC LABORATORY.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1902.





LETTER OF TRANSMITTAL.

SIR : I have the honor to transmit herewith a syllabus of the course in pathology and bacteriology given in this laboratory.

This is the completion of the third year in which the course of instruction as outlined in the following pages has been given by the present director of the laboratory to the student-officers of the Service. Advantage has been taken of many practical points gained from actual experience both from the standpoint of the student and that of the instructor, so that the course as now outlined is believed to fairly cover the ground and to require few changes other than those due to advances in our knowledge upon the subjects treated.

The object of the course is to thoroughly equip officers of the Service in the technique of pathology and bacteriology so as to fit them better for the hospital, quarantine, epidemic, and public-health work of the Service. Officers of the Service who have taken the prescribed course are thoroughly prepared to make the scientific diagnoses of plague, cholera, diphtheria, tetanus, tuberculosis, typhoid fever, anthrax, and other communicable diseases, as well as to carry on original investigations for themselves.

The course as outlined requires about one year for its completion. The student-officer is required to work each subject until he has mastered it before going on to the next. Such deliberation permits a thoroughness which is not possible when work is hurried or limited to a short space of time.

The exact sequence as given on the following pages is not strictly adhered to, because it is the desire of the director to make the course as practical as circumstances will permit. Therefore, the study of diphtheria is usually left until the fall of the year or winter, when practical experience may be had with the cases which usually occur in the District. The studies upon vaccinia and smallpox are taken up in the winter, which time is more apt to offer material and cases. Malarial work is of course confined to the summer and fall seasons. In connection with the health department of the District, the work of diagnosing diphtheria from swabs and culture tubes and the administration of anti-toxin, both for prophylactic and curative purposes, is carried on by each student-officer. In connection with the studies upon vaccinia, a visit is always made to one of the large vaccine farms, which offers special advantages for observing the process of vaccinating the calves, obtaining the lymph and pulp, glycerinizing the virus, and preparing

the product for the market. In like manner advantage is taken, as far as it is practicable, of the material in hospitals and other institutions situated in the city of Washington.

It will be noticed that the course apparently deals more with the subject of bacteriology than that of pathology. The latter, however, is not neglected. Owing to circumstances, it is not possible at present to treat pathologic-histology in the same systematic manner as bacteriology. The pathologic-histology of all the infectious processes studied in the laboratory are thoroughly worked. Tumors and the special pathology of the organs are taken up from time to time as the specimens are received. Experience has taught that this is better than to attempt to give a systematic course in general and special pathology, in which branches the student-officer usually comes to the laboratory with a good foundation.

It will also be noticed that considerable importance is given to the subject of disinfections and disinfectants. This arises from the fact that officers of the Service are required not only in their hospital work, but more especially in their quarantine and public health duties, to thoroughly understand the theory and practice of this important subject. Each student-officer is required to master the theory and mechanics of all the important disinfecting apparatus and to make actual tests upon the efficiency and practical value of each one.

The scientific workers in the laboratory meet one evening in each week in order to review the work of the week and to discuss the current literature. The journals that are received are assigned to each student-officer, and it is his duty to present a critical review of important articles which he reads. We have found this an admirable plan for keeping thoroughly abreast with the large amount of literature constantly appearing, as well as a stimulus to thought and work.

After the student-officer has been in the laboratory several months and has thoroughly acquainted himself with the necessary technique, opportunity is given him to assist with the work of the laboratory, and every encouragement is offered to work out original problems of his own.

The classes have been small in number, thus allowing for the individual equation of each student-officer, as well as a maximum of personal attention.

The bacteriologic nomenclature adopted in this bulletin is based upon Migula's recent *System der Bakterien*, 1900, and I am indebted to Dr. Ch. Wardell Stiles for valuable assistance in determining many points in this difficult and much-neglected subject.

Respectfully,

M. J. ROSENAU.

Passed Assistant Surgeon and Director Hygienic Laboratory.

THE SURGEON-GENERAL.



LABORATORY COURSE IN PATHOLOGY AND BACTERIOLOGY.

HYGIENIC LABORATORY,

United States Public Health and Marine-Hospital Service, Washington, D. C.

[By Milton J. ROSENAU, Passed Assistant Surgeon, United States Public Health and Marine-Hospital Service,
and Director of the Hygienic Laboratory.]

PREPARATION OF MEDIA—

1. Bouillon :
 - a. Acid.
 - b. Neutral.
 - c. Alkaline.
2. Sugar free bouillon (colon fermentation).
3. Glucose bouillon.
4. Lactose bouillon.
5. Dunham's solution.
6. Gelatin.
7. Agar-agar :
 - a. Glycerin agar.
 - b. Litmus agar.
8. Potato.
9. Blood-serum :
 - a. Fluid.
 - b. Coagulated.
 - c. Löffler's blood-serum mixture.
10. Milk.
11. Litmus milk.
12. Aqueous humor of ox eyes.
13. Ascitic fluid.
14. Urine.

STUDIES FOR MICROSCOPIC PRACTICE—

1. Diatoms.
2. Algae.
3. Protozoa.
4. Starch bodies.
5. Vegetable cells.
6. Cotton fibers.
7. Linen fibers.
8. Silk fibers.
9. Feathers.
10. Hair of man and of lower animals.
11. Scales of butterflies and of other insects.
12. Milk.
13. Air bubbles.
14. Oil drops.
15. Vinegar eels (*Anguillula aceti*).
16. Tube casts.
17. Ciliated epithelium.
18. Crystals of sugar, salt, uric acid, cholesterin, hematin, and various chemicals.
19. Decomposing meat.
20. Hay infusion.
21. Surface waters.



POINTS TO BE OBSERVED IN DESCRIBING AN ORGANISM—

1. Its source.
2. Hanging drop. Form, size, grouping and motility.
3. Spores.
4. Staining peculiarities.
5. Flagella.
6. Appearance of colonies on gelatine, agar, blood-serum, potato, etc.
7. Relation to oxygen.
8. Growth on gelatin.
9. Growth on agar.
10. Growth on blood-serum.
11. Growth on potato.
12. Growth on fluid media:
 - a.* Bouillon—
 1. Acid.
 2. Neutral.
 3. Alkaline.
 - b.* Dunham's solution—indol.
13. Milk and litmus milk.
14. Reaction of media best suited to growth.
15. Changes in reaction of media.
16. Gas production :
 - a.* Glucose bouillon.
 - b.* Lactose bouillon.
 - c.* Glucose agar.
 - d.* Glucose gelatin.
17. Temperature best suited to development.
18. Pathogenicity.
19. Reaction to sera of immune animals.
20. Toxins.

STUDY OF SAPROPHYTES.

BACTERIA.

Genus *Bacillus* Cohn—

1. *Bacillus subtilis* (Ehrenberg, 1833) Cohn, 1872.
2. *Bacillus vulgaris* (Hauser, 1885) Migula, 1900. [*Proteus vulgaris*.]
3. *Bacillus megaterium* de Barry, 1884.
4. *Bacillus prodigiosus* (Ehrenberg, 1839) Flügge, 1886.

Genus *Pseudomonas* Migula—

5. *Pseudomonas fluorescens* Migula, 1900. [*Bacillus fluorescens liquefaciens* Flügge, 1886.]
6. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872; *Bacillus pyocyaneus* Gessard, 1882.]

Genus *Planosarcina* Migula—

7. *Planosarcina agilis* (Ali-Cohen, 1889) Migula, 1900. [*Micrococcus agilis* Ali-Cohen, 1889.]

Genus *Sarcina* Goodsir—

8. *Sarcina lutea* Schröter, 1886.

Genus *Microspira* Schröter, 1886—

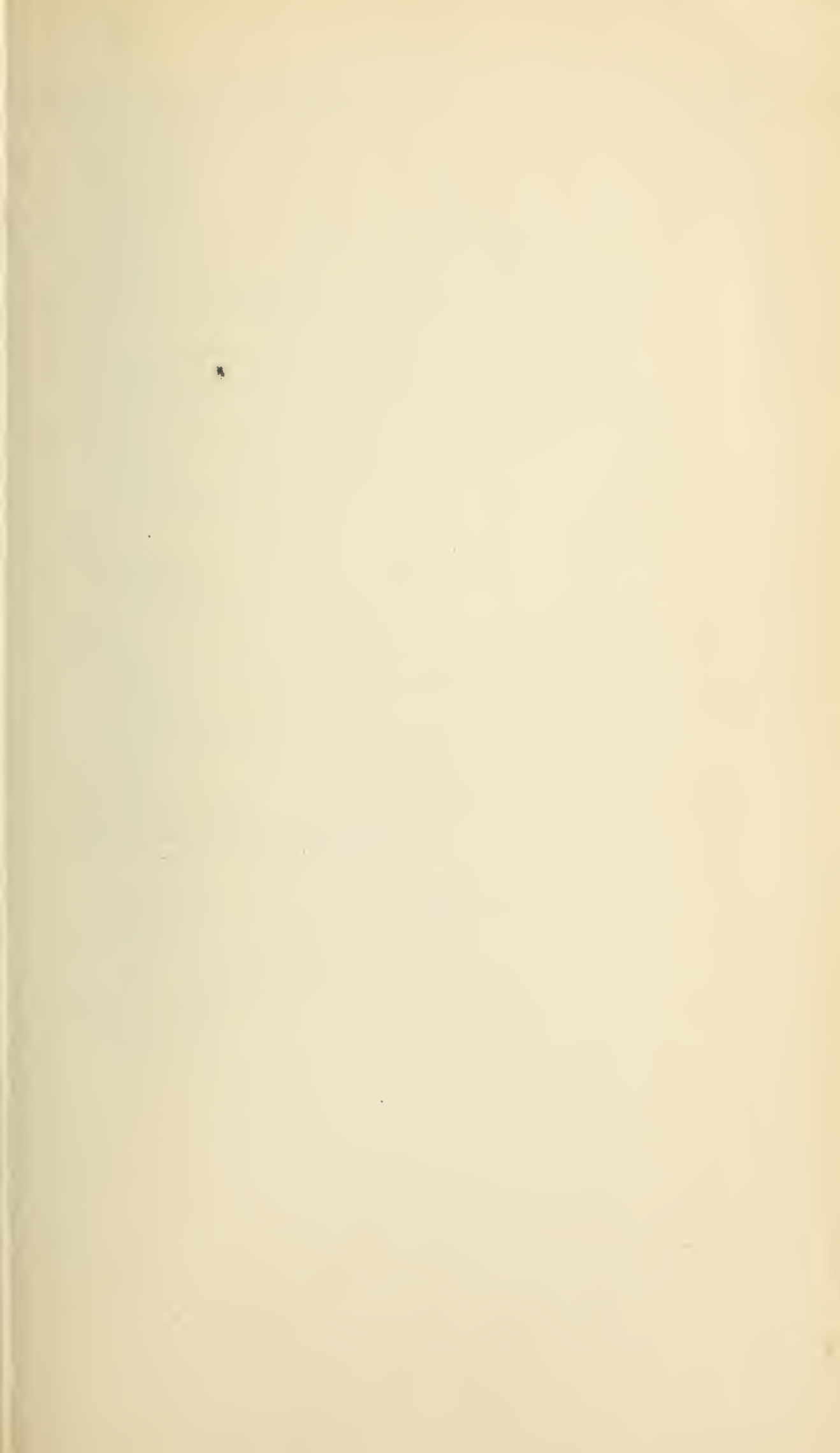
9. *Microspira aquatilis* (Günther, 1892) Migula, 1900. [*Vibrio aquatilis* Günther 1892.]

COMMON MOLDS—

Oidium.
Aspergillus.
Penicillium.
Mucor.

YEASTS—

Beer yeast.
 Red yeast.
 Black yeast.





CLASSIFICATION OF SCHIZOMYCETES—

1. According to some characteristic feature :

- a.* Chromogenic.
- b.* Photogenic.
- c.* Zymogenic.
- d.* Saprogenic.
- e.* Pyogenic.
- f.* Thiogenic.

2. According to morphology :

Cocci—

- a.* Micrococci.
- b.* Diplococci
- c.* Streptococci.
- d.* Staphylococci.
- e.* Tetrads.
- f.* Sarcinae.
- g.* Merismopedia.
- h.* Ascococcus.

Bacilli—

- a.* Leptothrix.
- b.* Cladothrix.
- c.* Streptothrix.

Spirilla—

- a.* Vibrio.
- b.* Spirillum.
- c.* Spirochaeta.

3. According to motion :

- a.* Nonmotile.
- b.* Motile.
- c.* Brownian motion.

4. According to arrangement of flagella :

- a.* Monotrichic.
- b.* Amphitrichic.
- c.* Lophotrichic.
- d.* Peritrichic.

5. According to relation to spores :

- 1. Nonspore bearing.
- 2. Spore bearing—
 - a.* Endospores.
 - b.* Arthrospores.

MAKING PLATES.

1. Koch's method—glass plates.
2. Esmarch's roll tubes.
3. Slants of agar and gelatin in test tubes.
4. Petri dishes—
 Streaking on surface with platinum wire.
 Daubing with swabs on surface.
 Mixing while fluid.

OBTAINING PURE CULTURES—

1. By plate method on solid media.
2. By dilution in liquid media.
3. By temperature.
4. Inoculation into animals.
5. Negative plates.
6. Special methods.

OBSERVE GROWTH AND MULTIPLICATION OF BACTERIA IN CELL SLIDE — AGAR METHOD :

1. Division of bacilli.
2. Division of cocci.
3. Sporulation.

STUDY OF BROWNIAN MOVEMENT WITH :

India ink and other substances in hanging drop.



THE PRINCIPLES, CHEMISTRY, AND PRACTICE OF STAINING—

A. Vegetable stains :

1. Carmine—

- a.* Acid.
- b.* Alcoholic.
- c.* Alum.
- d.* Borax.

2. Hematoxylin—

- a.* Delafield's hematoxylin.
- b.* Alum hematoxylin.
- c.* Hemalum (Meyer).
- d.* Hematoxylin and eosin.

B. Aniline stains :

1. Acid stains—

- a.* Eosin.
- b.* Acid fuchsin.
- c.* Orange G.
- d.* Soudan.
- e.* Picric acid.

2. Basic stains—

- a.* Methylene blue (Löffler's solution).
- b.* Thionin.
- c.* Fuchsin (Carbol-fuchsin).
- d.* Gentian violet (Ehrlich's aniline water.)
- e.* Methyl violet.
- f.* Bleu d' argent.

C. Gram's method and solution :

D. Decolorization and differentiation :

- a.* Water.
- b.* Alcohol.
- c.* Aniline oil.
- d.* Hydrochlorate of aniline, 2 per cent.
- e.* Acids—organic and mineral.

E. Staining of spores :

Möller's method.

F. Staining of flagella :

- a.* Löffler's method.
- b.* Victoria blue.
- c.* Von Ermengem's method.

PRINCIPLES, CARE, AND USE OF INSTRUMENTS AND APPARATUS—

- a.* Centrifuge.
- b.* Crematory.
- c.* Photographic apparatus.
- d.* Cleaning glass ware.
- e.* Microscope.
- f.* Paraffin bath.
- g.* Analytical balance.
- h.* Microtome.
- i.* Thermostats.
- j.* Thermo-regulators ; electric, mercurial, metallic.
- k.* Vacuum pumps.
- l.* Blow pipe.
- m.* Camera lucida.
- n.* Spectroscope.
- o.* X-ray tubes and induction coil.
- p.* Autoclaves.
- q.* Dry wall sterilizer.
- r.* Arnold steam sterilizer.
- s.* Blood serum sterilizer.
- t.* Cold chamber.
- u.* Sterilization of instruments.
- v.* Sharpening and care of cutting instruments.



OPTIC PRINCIPLES INVOLVED IN MICROSCOPY—

1. Lenses:
 - a.* Plano-convex.
 - b.* Biconvex.
 - c.* Concave.
 - d.* Biconcave.
 - e.* Doublets.
 - f.* Triplets.
 - g.* Quadruplets.
2. Objectives:
 - a.* Achromatics.
 - b.* Apochromatics.
 - c.* Panchromatics.
3. Oculars:
 - a.* Huyghenian.
 - b.* Compensation.
 - c.* Spectroscopic.
 - d.* Micrometer:
 - e.* Projection.
4. Condensers :
 - a.* Simple—
 1. Biconvex.
 2. Plano-convex.
 - b.* Abbe—
 - Numerical aperature 1.20.
 - Numerical aperature 1.40.
5. Microphotography and projection :
6. Micrometers :
 - a.* Stage.
 - b.* Ocular.
 - c.* Projection.

MEASURING MICROSCOPIC OBJECTS—

1. Eye-piece micrometer.
2. Stage micrometer.
3. Photographic method.

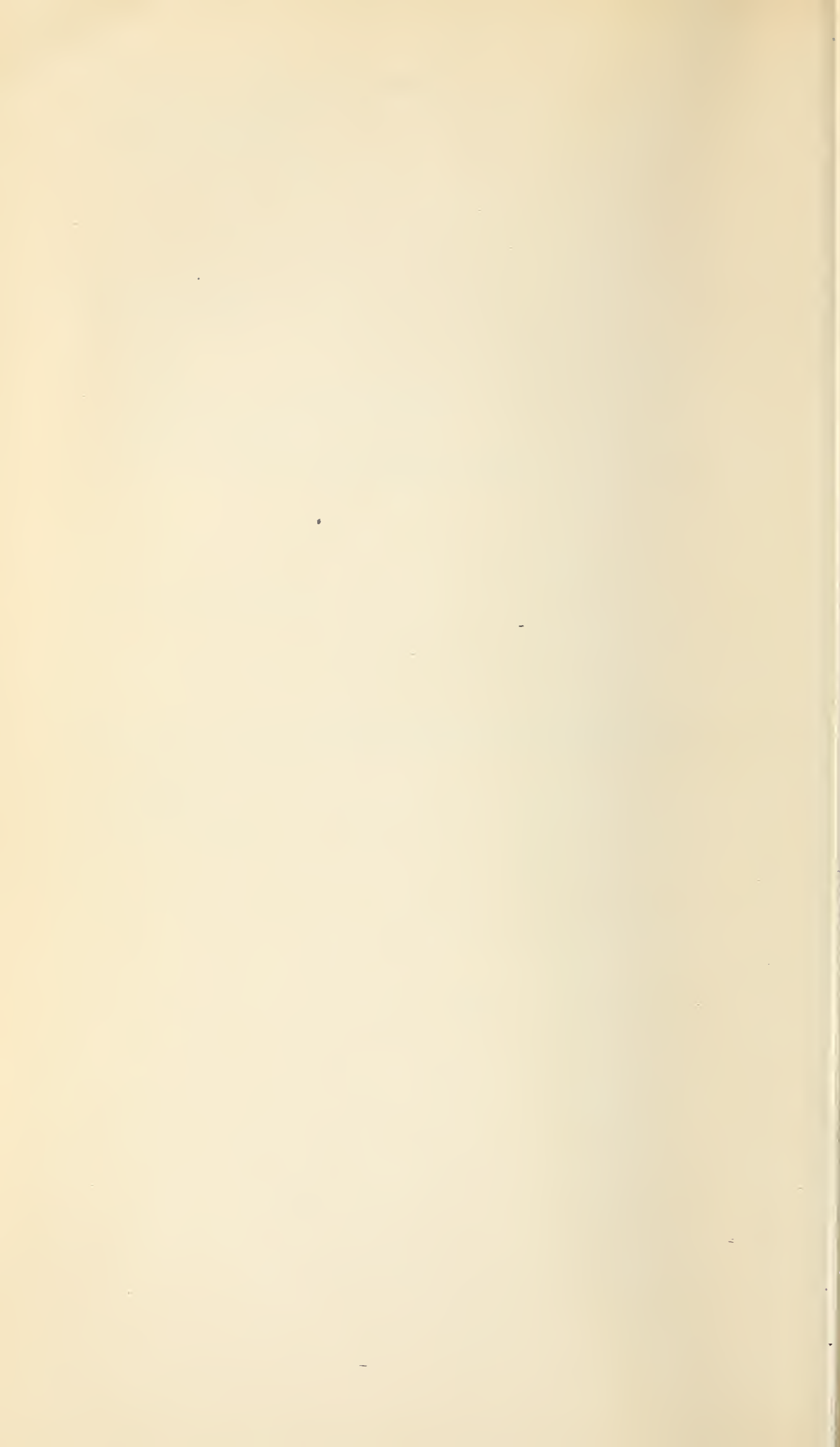
GLASS BLOWING—

1. The blowpipe.
2. Pipettes.
3. Bulbs.
4. Welding.
5. Sealing and opening glass containers.
6. Making test tubes.
7. Making perforated tubes for collodium sacks.

FOLDING PAPER FILTERS.

PREPARING BACTERIOLOGIC FILTERS (PRINCIPLES AND PRACTICE)—

1. Kaolin.
2. Diatomaceous earth.
3. Asbestos.
4. Sand filtration.

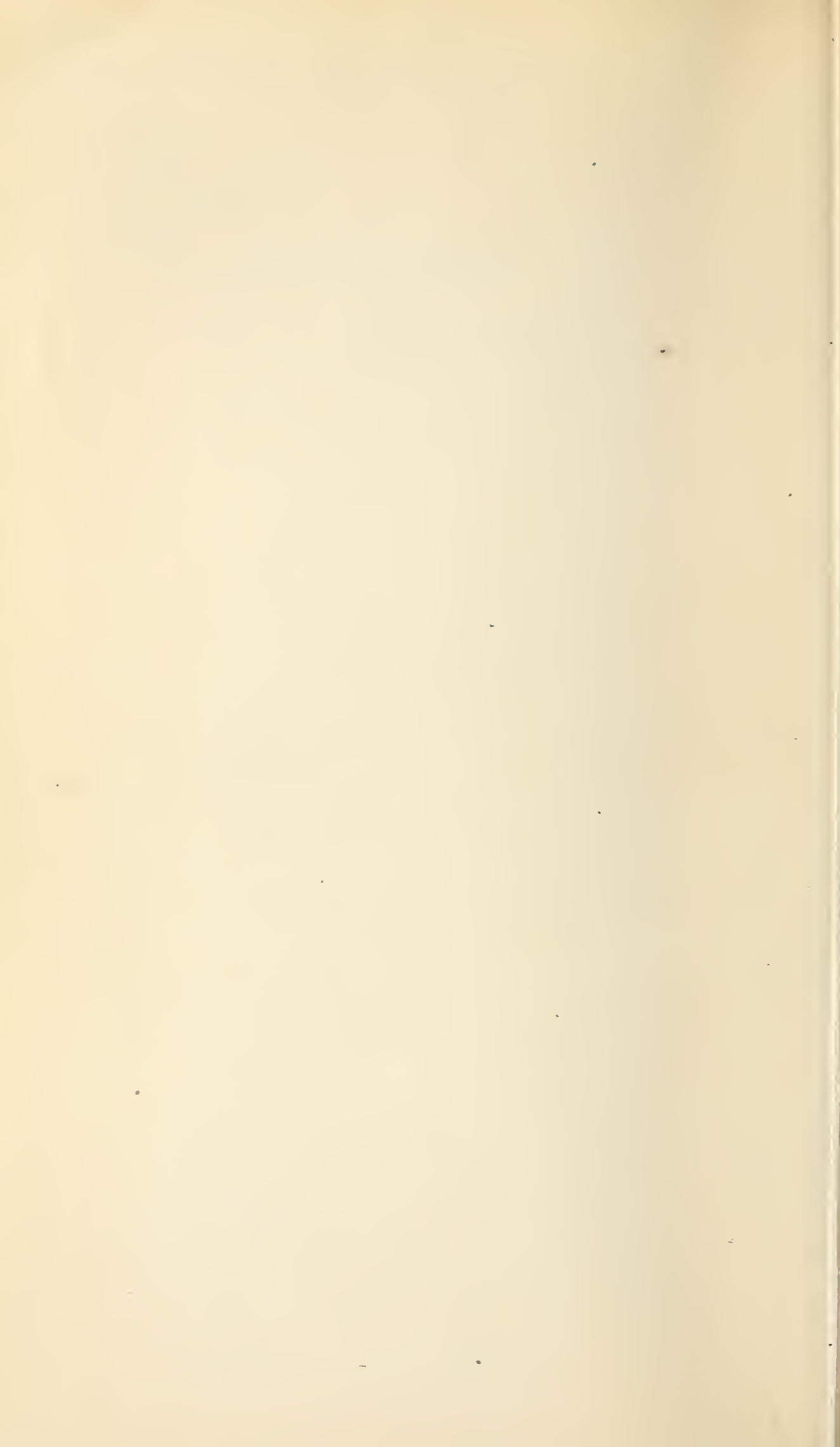


PREPARATION AND MOUNTING OF HISTOLOGIC SPECIMENS--

1. Fixation :
 - a.* Acetic-bichlorid of mercury.
 - b.* Müllers fluid.
 - c.* Chrom-acetic acid.
 - d.* Flemming's solution.
 - e.* Formaldehyd.
 - f.* Absolute alcohol.
2. Dehydration :
 - a.* Alcohol 70 per cent (iodine alcohol if Hg Cl_2).
 - b.* Alcohol 85 per cent.
 - c.* Alcohol 95 per cent.
 - d.* Absolute alcohol, twice.
3. Clearing :
 - a.* Xylol.
 - b.* Chloroform.
 - c.* Turpentine.
 - d.* Oil cloves.
 - e.* Oil bergamot.
 - f.* Oil origanum.
4. Infiltration and imbedding :
 - a.* Paraffin.
 - b.* Celloidin.
5. Sectioning :
 - a.* Single sections.
 - b.* Serial sections.
6. Attaching section to slide :
 - a.* Alcohol 85 per cent.
 - b.* Water.
 - c.* Myer's albumen.
 - d.* Gum Arabic.
 - e.* Collodium and clove oil.
7. Removal of imbedding material :
 - a.* Xylol to remove paraffin.
 - b.* Alcohol and water.
8. Staining :
 - a.* Nuclear staining.
 - b.* Protoplasmic.
 - c.* Regressive.
 - d.* Gram's.
 - e.* Bacterial.
 - f.* Special.
9. Dehydration in absolute alcohol, aniline oil :
10. Clearing :
 - a.* Xylol.
 - b.* Turpentine.
 - c.* Oil origanum.
 - d.* Oil bergamot.
 - e.* Oil cloves.
 - f.* Chloroform.
11. Mounting in xylol balsam :

THE PARAFFIN BATH AND PARAFFIN IMBEDDING—

- A. Fixing.
- B. Dehydration.
- C. Clearing.
- D. Imbedding in paraffin.
- E. Molding block.
- F. Cutting.
- G. Method of numbering, registering, and storing.



CELLOIDIN IMBEDDING—

- A. Prepare a thin celloidin solution containing 4 per cent celloidin in equal parts of absolute alcohol and ether.
- B. Prepare a saturated solution in the same solvent.
- C. Transfer tissue from absolute alcohol to equal parts of alcohol and ether.
- D. From alcohol and ether to thin celloidin.
- E. From thin celloidin to thick celloidin.
- F. From thick celloidin to paper mold on cork.
- G. Into 70 per cent alcohol for three days to harden.
- H. Cut section with knife flooded with 70 per cent alcohol.
- I. Stain.
- J. Dehydrate.
- K. Clear.
- L. Mount.

METHOD OF MAKING FROZEN SECTIONS. (Wright.)

1. Place tissue not over 5 mm. thick in 10 per cent formalin for two or more hours.
2. Wash in water.
3. Freeze with CO_2 or ether.
4. Cut frozen sections.
5. Float sections in normal salt solution and then on slide.
6. Cover section with cigarette paper, then with blotter moistened with 95 per cent alcohol. Press. Remove papers.
7. Cover with absolute alcohol thirty seconds. Drain.
8. Flow over the section and adjacent parts of the slide a thin solution of celloidin in equal parts of absolute alcohol and ether. Drain off immediately. The celloidin should form a coating so thin as to be invisible.
9. Flood the slide with 95 per cent alcohol and then immerse in water. This hardens the film.
10. Stain.
11. Dehydrate.
12. Clear with oil origanum.
13. Mount in Canada balsam.





STAINING TISSUES IN SECTIONS.

1. Hem-alum and eosin.
2. Delafield's hematoxylin.
3. Eosin and thionin.
4. Carmine stains.
5. Weigert's fibrin stain.
6. Jenner's stain.
7. Van Giesen for connective tissues.

STAINING BACTERIA IN SECTIONS.

1. Gram's method.
2. Eosin and thionin.
3. Thionin.
4. Methylene blue.

STUDY OF TUMORS.

Classification.

Cut sections and study :

1. Myomata.
2. Neuromata.
3. Angiomata :
 - a. Simple.
 - b. Cavernous. ●
4. Lymphangiomata :
 - a. Simple.
 - b. Cavernous.
5. Fibromata.
6. Myxomata.
7. Lipomata.
8. Chondromata.
9. Osteomata.
10. Gliomata.
11. Sarcomata :
 - a. Round celled.
 - b. Lympho-sarcoma.
 - c. Alveolar.
 - d. Spindle celled.
 - e. Melano sarcoma.
 - f. Osteoid.
 - g. Psammomata.
 - h. Myeloid.
 - i. Angio sarcoma.
 - j. Endothelioma.
12. Papillomata.
13. Adenomata.
14. Carcinomata :
 - a. Acinous.
 - b. Epitheliomata.
15. Cystomata.

ANIMAL INOCULATIONS—

1. Subcutaneous method :
 - a.* Liquids.
 - b.* Solids.
2. Intravenous method.
3. Inoculation into lymphatic system.
4. Inoculation into serous cavities :
 - a.* Peritoneum.
 - b.* Pleural cavity.
 - c.* Subdural space.
5. Inoculation into anterior chamber of eye.

OBSERVATION OF ANIMALS AFTER INOCULATION—

1. Temperature.
2. Loss of weight.
3. Peculiar position in cage.
4. Loss of appetite.
5. Condition of coat.
6. Condition of secretions, etc.

POST-MORTEM EXAMINATION OF ANIMALS—

1. Cultures from the organs.
2. Cultures from the fluids.
3. Preparation and staining of tissues.

INCINERATION OF DEAD ANIMALS AFTER EXAMINATION—

MAKING COLLODIUM SACKS—

- Paris method.
- Gelatin capsule method.
- With glass support and gelatin (method of Grubbs & Francis).

BACILLUS COLI Migula, 1900. [*Bacterium coli commune* Escherich.]

- A. Distribution.
- B. Isolate from sewage.
- C. Characteristics:
 - 1. Motility.
 - 2. Gram's method.
 - 3. Nonliquefaction of gelatin.
 - 4. Indol.
 - 5. Gas production.
 - 6. Colonies, etc.
- D. Pathogenesis.
- E. Varieties.
- F. Preparation of its toxin.
- G. Pathogenicity.
- H. Study of blood and organs of infected animals.
- I. Significance in drinking water.
- J. Viability.

BACILLUS ANTHRACIS Koch. 1876.

A. History.

B. Distribution.

C. Morphology:

1. Involution forms.

2. Asporogenous form.

D. Staining.

E. Cultural characteristics.

F. Spores:

1. Formation—

Relative to oxygen.

Relative to nutrient media.

Relative to temperature.

2. Development into bacteria.

G. Pathogenesis (mouse, guinea pig, and rabbit.)

a. Pneumonic.*b.* Hemorrhagic.*c.* Local.

H. Examination of liver, blood, spleen, lungs, and other organs.

I. Vaccination:

First vaccine—kills mice, not guinea pigs or rabbits.

Second vaccine—kills mice and guinea pigs, not rabbits.

Third vaccine—kills mice and guinea pigs and some rabbits.

J. Disposal of anthrax carcasses:

a. Burial, isolated districts.*b.* Burning.*c.* Sulphuric acid.*d.* Boiling.

K. Human anthrax:

a. Malignant pustule.*b.* Wool sorter's disease.*c.* Intestinal form.

L. Viability.

M. Disinfection for anthrax.

BACTERIUM DIPHTHERIÆ (Löffler) Migula. [*Klebs-Löffler Bacillus.*]

- A. History.
- B. Morphology—relation to clinical types.
- C. Staining :
 - 1. Löffler.
 - 2. Neisser.
 - 3. Roux.
 - 4. Weigert's fibrin method for tissues.
 - 5. Gram's stain.
- D. Biologic characteristics.
- E. Pathogenesis :
 - 1. Guinea pig.
 - 2. Rabbit.
 - 3. Kitten.
- F. Toxin production.
- G. Antitoxin production :
 - 1. Standardization.
 - 2. Prophylactic use.
 - 3. Curative use.
- H. Obtain tubes and swabs from the health department for diagnosis.
 - I. Pseudo-diphtheria bacillus.
- K. Xerosis bacillus.
- L. Viability.
- M. Disinfection for diphtheria.

BACTERIA IN INFLAMMATION AND SUPPURATION—

Study :

- A. *Micrococcus aureus* (Rosenbach, 1884) Migula, 1900. [*Staphylococcus pyogenes aureus* Rosenbach, 1884.]
- B. *M. pyogenes* (Rosenbach, 1884) Migula, 1900. [*S. pyogenes albus* Rosenbach, 1884.]
- C. *M. citreus* (Passet, 1885) Migula, 1900. [*S. pyogenes citreus* Passet, 1885.]
- D. “*Staphylococcus epidermidis albus* Welch.”
- E. *M. gonorrhææ* (Bumm, 1885) Flügge, 1886.
- F. *Streptococcus pyogenes* Rosenbach, 1884.
- G. “*Streptococcus erysipelatos* Rosenbach,” 1884
- H. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872 ; *Bacillus pyocyaneus* Gessard, 1882.]

Collect pus and inflammatory exudates from various sources, plate out and study bacteria found.

Study pus in smears on slides.

Ducrey's bacillus—Soft chancre.

Micrococcus melitensis Bruce, 1893.

THE TYPHOID GROUP—

Study :

- A. *Bacillus typhosus* Migula, 1900.
- B. *Bacillus dysenteriae* Shiga, 1898 [not Migula, 1900].
- C. *Bacillus enteritidis* Gaertner, 1888.
- D. Para-colon bacillus.
- E. Para-typhoid bacillus.
- F. *Bacillus murium* Migula, 1900. [*Bacillus typhi murium* Löffler, 1893.]
- G. Bacillus of Danyz's virus.
- H. *Bacillus psitticosis*.
- I. *Bacillus icteroides* Sanarelli, 1897.
- J. *Bacillus coli* Migula, 1900. [*Bacterium coli commune* Escherich.]
- K. Methods of introduction into the system.
- L. Channels of elimination.
- M. Viability.
- N. Disinfection.
- O. Relation to the water supply.
- P. Relation to flies and other insects.
- Q. Widal reaction.—
 - a. With hanging drop.
 - b. In pipettes.

THE TUBERCLE GROUP—

A. *Bacillus tuberculosis* Koch, 1882 :

1. Human. Isolate in pure culture from sputum.
2. Bovine.
3. Avian.

Tuberculin—

Production.

Uses :

1. Diagnostic.
2. Therapeutic.

B. Acid proof organisms, resembling *B. tuberculosis* :

1. Möller's grass bacillus. Isolate from timothy.
2. Rabinowitsch's butter bacillus.
3. Karlinsky's nasal secretion organism.
4. Smegma bacillus.

C. *Bacillus lepræ* Hansen.D. *Bacillus mallei* (Löffler, 1886) Migula, 1900 :

Mallein.

Straus method in male guinea pig.

E. Actinomycosis.

F. Mycetoma, Madura foot.

G. Pathogenic streptothrices.

THE VIBRIO GROUP—

- A. *Microspira comma* Schröter, 1886. [Koch's comma bacillus of Asiatic cholera.]
- B. *Microspira Metshnikovi* (Gamaleia, 1888) Migula, 1900.
- C. *Microspira Finkleri* Schröter.
- D. *Vibrio Denecke*.
- E. *Microspira aquatilis* (Günther, 1892) Migula, 1900.
 - Pfeiffer's phenomenon.
 - Widal reaction.
 - Immunity.
 - Haffkine's prophylactic.
 - Viability.
 - Disinfection for cholera.

RELAPSING FEVER.

BACILLUS PESTIS Kitasato & Yersin, 1894.

A. Study—morphology, staining and cultural characteristics :

Viability.

Channels of entrance.

Channels of elimination.

Pathogenicity—guinea pigs, rabbits, rats, mice.

Diagnosis :

Yersin serum—

Method of manufacture and standardization.

Prophylactic use.

Curative use.

Haffkine prophylactic—

Methods of manufacture.

Uses.

B. Disinfection.

C. Quarantine.

D. Relation of rats and other mammals to spread of plague.

E. Relation of fleas, flies, and insects to spread of plague.

SEPTICEMIC GROUP—

- A. Mouse septicemia.
- B. Rouget du porc.
- C. Chicken cholera.
- D. Swine plague.
- E. Ferret, duck, buffalo, and other plagues.
- F. *Bacillus pestis* Kitasato & Yersin, 1894.
- G. *Bacterium pneumoniæ* (Weichselbaum, 1886) Migula, 1900. [*Diplococcus pneumoniæ* Weichselbaum, 1886.]
- H. *Bacterium pneumonicum* Migula, 1900. [*Pneumonicoccus* Friedländer, 1882.]
- I. *Bacterium influenzæ* Lehmann & Neumann, 1896.

ANAËROBIC TECHNIQUE—

- A. Kipps' hydrogen apparatus :
 - 1. Mechanics.
 - 2. Chemicals.
- B. Simple hydrogen generators.
- C. Solutions for purifying gas :
 - 1. Silver nitrate for chlorine.
 - 2. Potassium permanganate for oxygen.
 - 3. Lead acetate for sulphides.
- D. Media best suited for anaërobes :
 - 1. Freshly prepared media.
 - 2. Glucose media.
- E. Special methods :
 - 1. Fraenkel's method.
 - 2. Esmarch's method.
 - 3. Liborius' method.
 - 4. Buchner's method.
 - 5. Novy's jars.
 - 6. Wright's method.
 - 7. Pasteur Institute method.
 - 8. Vignal's tubes.
 - 9. Full tubes of bouillon.
- 10. Deep stabs.
- 11. Oil method.

ANAËROBES—

- A. *Bacillus tetani*, its toxin and antitoxin.
- B. *Bacillus œdematis* of malignant edema.
- C. *Bacillus Chauvæi* Arloing, Cornevin & Thomas, of symptomatic anthrax :
Vaccine—
 - 1. Manufacture.
 - 2. Use.
- D. *Bacillus Welchii* Migula, 1900. [*Bacillus aerogenes capsulatus* Welch & Nuttall, 1892.]
Disinfection for above.

PATHOGENIC MOLDS—

*Ring worm:**Microsporon Audouini* (Gruby).*Trichophyton*—1. *T. endothrix*.2. *T. ectothrix*.

Hair lesions.

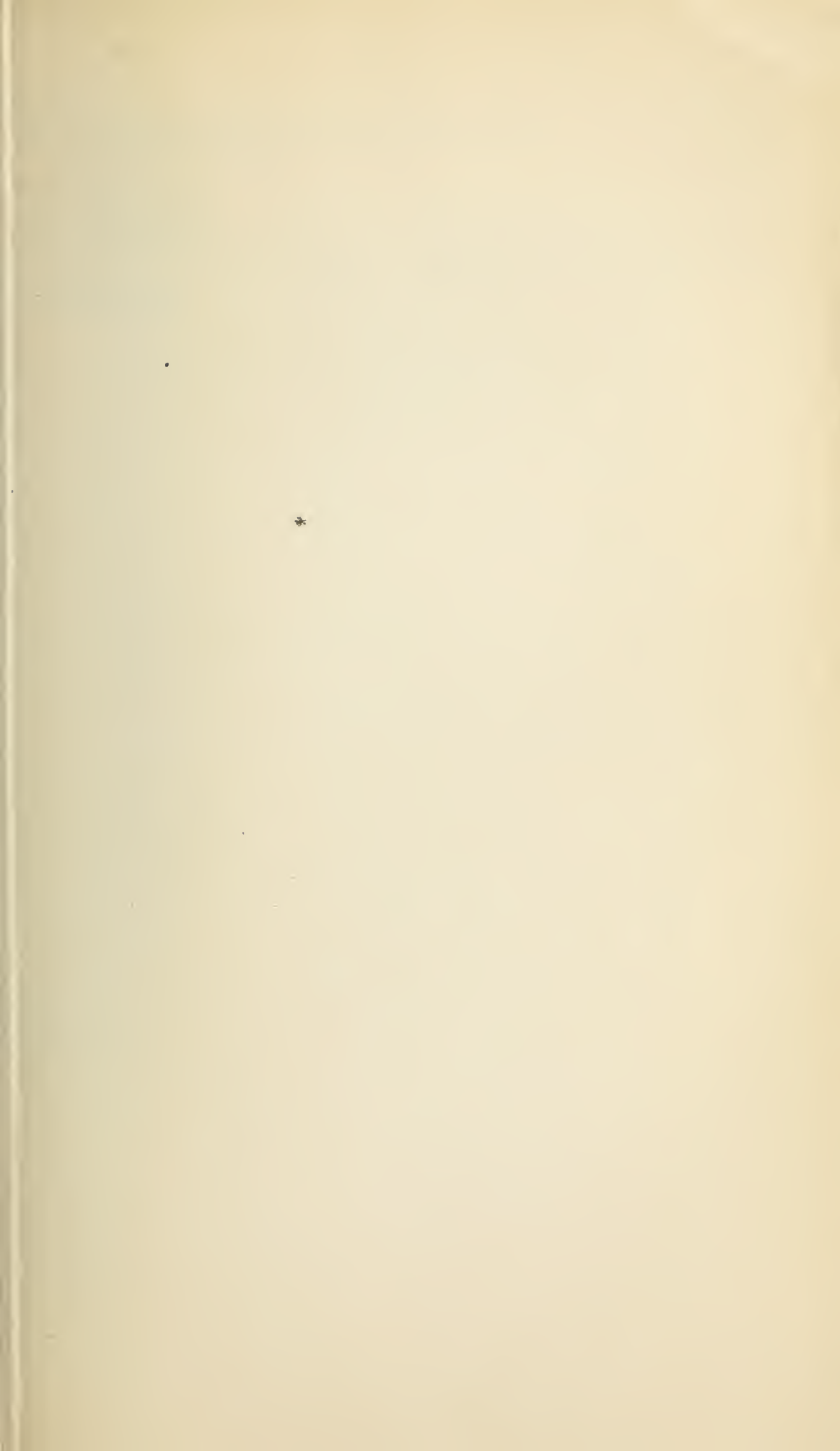
Pure cultures.

Favus:

Achorion Schoenleinii Remak.

In the hair and scale.

In pure culture.



YEASTS—

Saccharomyces cerevisiæ Meyen (beer yeast) in pure cultures :

Effect on saccharine media.

Budding.

Red yeast—*S. glutinis*.

Black yeast—*S. niger*.

Saccharomyces albicans (Robin) Reess, 1877 [*Oidium albicans* Robin] thrush, in pure culture.

Pathogenic yeasts.

EXAMINATION OF URINE—

Note :

1. Odor.
2. Color.
3. Deposit. Filtration of specimen.
4. Specific gravity.
5. Reaction.
6. Quantity in twenty-four hours.
7. Albumen—

Tests for albumen ; quantitative and qualitative :

- a.* Heat (phosphates).
- b.* Nitric acid, in test tube and in glass.
- c.* Ferro-cyanide potash ; acetic acid.
- d.* Picric acid, Esbach's albumenometer.

8. Sugar—

Test for sugar :

- a.* Fehling's solution.
- b.* Bismuth.
- c.* Quantitative estimation with yeast and with Fehling's solution.

9. Estimation of urea.
10. Bile, test for.
11. Microscopic examination of sediment for bacteria, casts, crystals, blood, etc.
Schistosoma hæmatobium (Bilharz, 1852) Weinland, 1858.
12. Test for chlorides.
13. Diazo reaction.
14. Bacteriologic examination of urine.
15. Disinfection of urine.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF FECES—

1. Microscopic :

a. Normal constituents.

b. Intestinal parasites and their eggs—

Ascaris lumbricoides (Linnæus, 1758).*Oxyuris vermicularis* (Linnæus, 1758) Bremser, 1819.*Trichuris trichiura* (Linnæus, 1771).*Uncinaria duodenalis* (Dubini, 1843).*Uncinaria americana* Stiles, 1902.*Tænia saginata* Gœze, 1782.*Tænia solium* Linnæus, 1758.*Dibothriocephalus latus* (Linnæus, 1758).*Amœba coli* Lœsch, 1875.

2. Bacteriologic :

a. Normal flora.

b. Pathogenic—

Bacillus dysenterix.*Bacillus tuberculosis*.*Bacillus typhosus*.*Microspira comma*.

3. Disinfection of feces.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF—

Sputum, nasal and other mucous secretions :

Tubercle bacilli.

Pneumococci.

Influenza bacterium.

Disinfection of sputum.

BLOOD.

Technique of:

1. Collecting blood for microscopic examination.
2. Collecting blood for bacteriologic examination—
 - a.* Small amount.
 - b.* Large quantities.
 - Of man.
 - Of other mammals.
 - Of birds.
 - Of reptiles.
3. Observe circulation of blood under microscope.
 - Mesentery of frog.
4. Estimate alkalinity.
5. Estimate specific gravity of blood with mixture of chloroform and benzol.
6. Examine fresh blood in hanging drop.
7. Examine fresh blood between slide and cover slip.
8. Study of blood films :
 - a.* Method with cover glass.
 - b.* Method with special rod.
 - c.* Method with cigarette paper.
9. Fixation of blood films :
 - a.* Heat, 150° C. for about five minutes.
 - b.* Alcohol and ether.
 - c.* Formalin.
 - d.* Formaldehyd.
 - e.* Acetic acid bichlorid.
10. Staining.
 - a.* Ehrlich-Biondi-tri-acid stain.
 - b.* Jenner's stain.
 - c.* Romanowsky's stain.
 - d.* Goldhorn's fluid.
 - e.* Eosin and methylen blue.
 - f.* Thionin and eosin.
11. Counting red corpuscles.
 - Thoma-Zeiss hemocytometer.
12. Estimating the number of leucocytes :
 - a.* With the Thoma-Zeiss hemocytometer.
 - b.* Comparative count of—
 - a.* Small lymphocytes.
 - b.* Large lymphocytes and transitional forms.
 - c.* Polymorphonuclear neutrophiles.
 - d.* Eosinophiles.
 - e.* Mast cells.
13. Estimating the amount of hemoglobin :
 - With—
 - a.* Dare's hemoglobinometer.
 - b.* Oliver's hemoglobinometer.
 - c.* Gower's hemoglobinometer.

BACTERIA IN THE AIR—

1. Number :

Sewer air.

Expired air.

Sea air.

City air.

Country air.

Air in houses, hospitals, etc.—

Relation to the sun.

Relation to habitation.

Relation to winds and traffic.

Relation to altitude.

Pasteur's method.

Miquel's method.

Aeroscopes.

Frankland's method.

Petri's method.

Hesse's method.

Straus' method.

2. Varieties.

3. Estimation of ammonia in the air.

4. Estimation of carbon dioxid in the air

BACTERIA IN WATER—

1. Count number per 1,000 c. c. :
 - a.* In tap water.
 - b.* In river water.
 - c.* In spring water.
 - d.* In rain water.
 - e.* In ice.
2. Varieties.
3. Collecting samples.
4. Estimation of organic matter—
 - a.* Free ammonia.
 - b.* Albuminoid ammonia.
5. Estimation of nitrates and nitrites.
6. Estimation of chlorids.
7. Purification of water :

Heat.

Filters—

- a.* Household filters.
- b.* Field and camp filters.
- c.* Sand filters—

Alum.

Hyperoxid of chlorin.

Ozone.

Permanganate of potash.

BACTERIA IN SOIL—

1. Number.
 2. Varieties.
- Nitrification.

PURIFICATION AND DISPOSAL OF SEWAGE.

1. Dilution.

This method is allowable for cities situated on the sea or on rivers whose flow is very large as compared with the sewage (100 : 1) and the unfiltered water of which is not used as a water supply.

2. Sewage farming.

3. Chemical precipitation.

4. Intermittent filtration.

Needs sandy soil.

5. Septic tank.

6. Contact bed treatment.

7. Continuous filtration :

a. Scott-Moncrieff system.

b. The Ducat.

c. Whitaker & Bryant.

d. F. Stoddart.

e. G. Salford.

MILK—

1. Histology.
2. Bacteria in milk :
 Number.
 Origin.
 Varieties.
 Diseases conveyed by milk.
3. Fermentation—
 Lactic acid.
 Butyric acid.
 Sweet curdling.
 Abnormal.
4. Pasteurization :
 Theory and practical uses.
 Changes produced in milk.
5. Count and study colonies in fresh milk and milk bought in open market.
 Pasteurize, then count and study colonies.
6. Sterilization of milk by heat; practical application and changes produced in milk.
7. Milk preservatives.

PRINCIPLES AND PRACTICE OF DISINFECTION AND STERILIZATION.

Physical agents.

1. Sunlight—

Expose spores of anthrax, subtilis, etc.

Expose nonspore-bearing organisms as typhoid, diphtheria, etc., to the sunlight in thin films and for penetration. Note intensity of the light, temperature, dryness, and other conditions. Plant in bouillon.

2. Electricity—

Expose slips and cultures to various currents.

Expose to electric light.

Expose to X-rays.

3. Dryness and dry heat—

Expose slips, or threads with spore-bearing and nonspore-bearing organisms to :

a. Dryness at room conditions.

b. Dry in incubator at 37° C.

c. Dry over sulphuric acid.

d. Dry heat at 60° C.,

100° C.,

150° C.,

for variable times.

e. Expose paper slips saturated with—

a. Decomposing urine,

b. Sewage,

c. Garden earth,

to 150° C. for one hour to test power of sterilization of this temperature.
Hot-air sterilizer.

NOTE.—Plant in bouillon and keep one week for growth.

5. Moist heat—hot and boiling water and steam :

Expose typhoid, diphtheria, and other nonspore-bearing organisms to 50°, 60°, 70°, 80°, and 100° C. moist heat for variable times ; plant in bouillon.

Expose anthrax, subtilis, and other spores to 70°, 80°, 90°, and 100° C. moist heat for variable times ; plant in bouillon.

6. Theory, mechanics, and practical application of steam in—

a. Koch steamer.

b. Arnold steam sterilizer.

c. Autoclave (steam under pressure).

d. Steam chamber (steam under pressure).

Make practical tests in each.

TESTING OF ANTISEPTICS—

If the substance belongs to the Cresol group, prepare 1 per cent, 2 per cent, 3 per cent, 5 per cent, 7 per cent, 9 per cent, 10 per cent solutions of the substance in a set of bouillon tubes.

If the substance belongs to the metallic salts, make $\frac{1}{5000}$, $\frac{1}{3000}$, $\frac{1}{2000}$, $\frac{1}{1000}$, $\frac{1}{250}$, etc., solutions in a set of bouillon tubes.

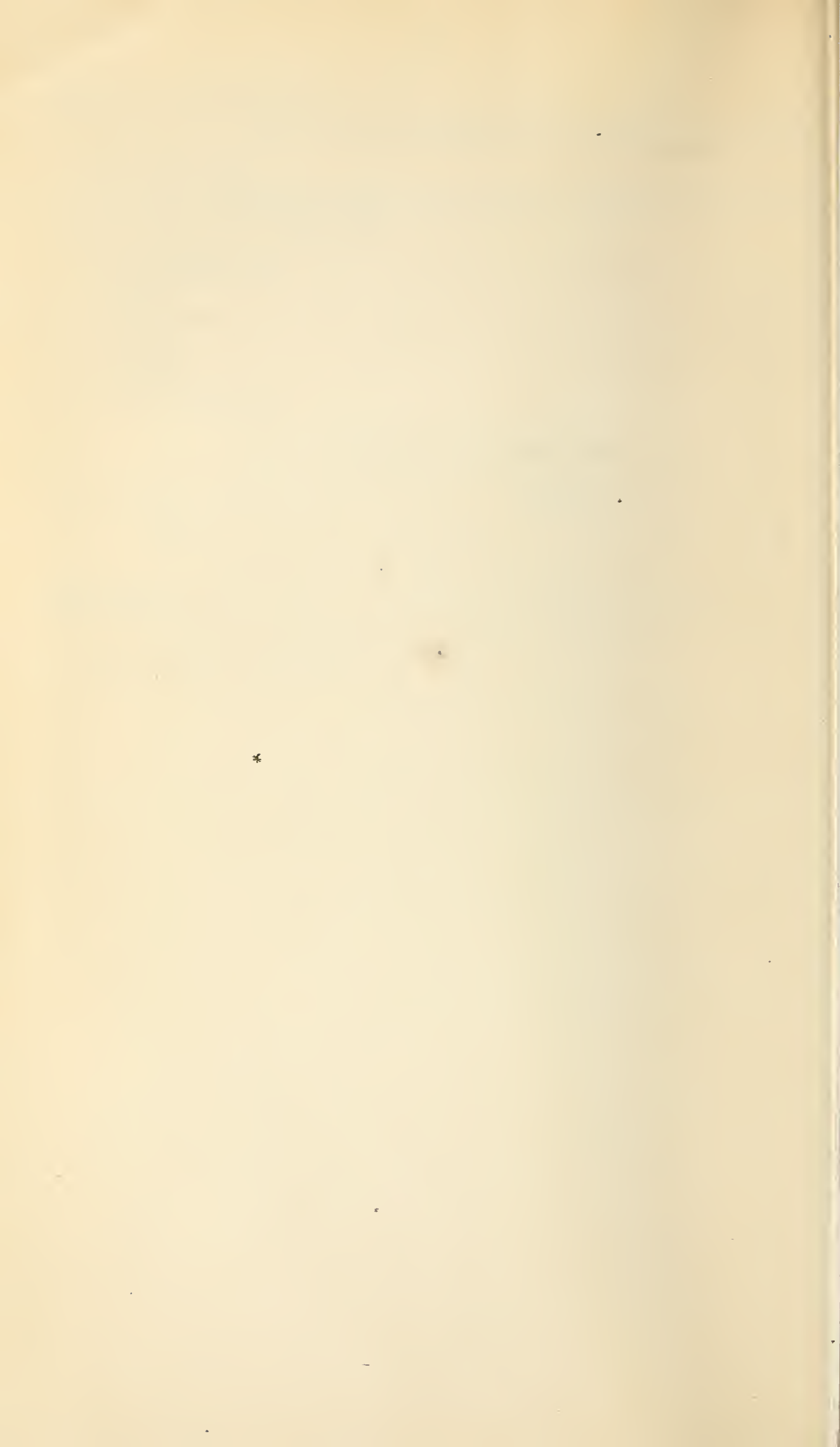
Inoculate with :

- a. Decomposing urine.
- b. Sewage.
- c. Garden earth.
- d. Typhoid.
- e. Pus cocci.
- f. Anthrax spores, etc.

Incubate and make report on the following plan :

Name of substance.—Strength of solution.	Minutes.							
	1.	2.	3.	5.	10.	15.	20.	30.
Sewage	—	—	—					
Garden earth	—	+	+					
Decomposing urine.....	+	+	+					

— means no growth, + means growth.



TESTING GERMICIDES—

Saturate silk threads,
 paper slips,
 glass slips, etc., with
 subtilis spores,
 anthrax spores,
 typhoid bacillus,
 diphtheria bacillus,
 pus cocci, etc.

Expose to the solutions of germicide in varying dilutions and for varying times at constant temperature, and at the same dilution at varying temperatures.

Wash or neutralize the germicide.

Plant in bouillon.

Incubate.

Report growth upon similar table to antiseptics.

Test the following upon the above plan—

- A. Bichloride of mercury.
- A. Carbolic acid and cresols.
- C. Lime.
- D. Copper and iron sulphates.
- E. Formalin.

GASEOUS DISINFECTION—

A. Formaldehyd:

- a.* Chemistry and physics.
- b.* Sheet method.
- c.* Generator—
 - 1. Kuhn lamp.
- d.* Regenerators without pressure—
 - 1. Trenner-Lee.
 - 2. Lentz.
- e.* Regenerators with pressure—
 - 1. Autoclave.
- f.* Heating paraform.
- g.* Spraying.

B. Sulphur dioxid (SO_2):

- a.* Liquid sulphur dioxid.
- b.* Burning sulphur, pot method.
- c.* Sulphur furnace.

C. Hydrocyanic acid gas.

D. Chlorin gas.

E. Ozone and oxygen.

INSECTICIDES—

- A. Tobacco smoke.
- B. Pyrethrum.
- C. Hydrocyanic acid.
- D. Formaldehyd gas.
- E. Sulphur dioxide.
- F. Coal oil.
- G. Arsenical compounds.
- H. Phosphorus.
- I. Cobalt salts.
- J. Carbon bisulphid for mosquitoes, adults, pupæ, and larvæ :
 - Ants,
 - Mosquitoes,
 - Roaches,
 - Fleas,
 - Flies, etc.

1. THE DISINFECTION OF HOUSES, SHIPS, AND OBJECTS.
2. THE DISINFECTION AND PROPHYLAXIS AGAINST THE COMMUNICABLE DISEASES, ESPECIALLY :
 - a.* Yellow fever.
 - b.* Cholera.
 - c.* Plague.
 - d.* Smallpox.
 - e.* Tuberculosis.
 - f.* Typhoid fever.
 - g.* Malaria.
 - h.* Dysentery.
 - i.* Diphtheria.
 - j.* Pneumonia.
 - k.* Leprosy.
 - l.* Anthrax.
 - m.* Tetanus.
 - n.* Dengue.
 - o.* Relapsing fever.
 - p.* Typhus fever
 - q.* Scarlet fever.
 - r.* Measles.

Including channels of introduction and elimination.

Viability.

Disinfection.

Prophylaxis.

Management of epidemics.

Quarantine.

RABIES—

Study in dogs.

Study in rabbits.

Prepare spinal cords for preventive inoculation.

Principles and methods of Pasteur treatment.

VACCINATION (*vaccinia*)—

- A. History.
- B. Methods of production :
 - Vaccine lymph.
 - Vaccine pulp—
 - Dry points.
 - Glycerinated pulp.
- C. Technique of vaccination.
- D. Immunity produced.
- E. Bacteriologic and microscopic study of vaccine matter.
- F. Bodies in epithelial cells—cornea of rabbits.

Examination of vaccine—

- G. Note the name of maker, kind of virus, etc.
- H. Count the number of colonies upon :
 - 1. Three glycerinated tubes upon agar.
 - 2. Three glycerinated tubes or points upon gelatin.
 - 3. Three dry points upon agar.
 - 4. Three dry points upon gelatin.
- I. Study the character of the organisms found upon media and animals.
- J. Plant the entire contents of 6 tubes in bouillon, grow anaërobically and study the characters of the growth upon media and by inoculation in animals.
- K. Study the dry points as in J.
- L. Inoculate the contents of 12 tubes and 12 points directly into mice, rats, and guinea pigs.
- M. Keep full notes of all results.
- N. Visit to the vaccine farm for practical study of manufacture of vaccine commercially.

MALARIA—

1. Diagnosis.
2. Blood technique.
3. Relation to mosquito :
 - a. Tertian.
 - b. Quartan.
 - c. Aestivo-autumnal.

Mosquitoes—

Anatomy—dissections fresh specimens.

Sections in paraffin.

Development and raising.

Classification.

Diagnosis, especially :

- a. *Culex*.
- b. *Anopheles*.
- c. *Stegomyia*.

A. Malaria—the *Anopheles* :

Study *Anopheles* one each day after biting a malarial case.

Tertian.

Quartan.

Estivo-autumnal.

B. Yellow fever and the mosquito.

C. *Filaria* and the mosquito.

INSECTS AS FACTORS IN TRANSMITTING DISEASE—

The mosquito.

The fly.

The ant.

The flea.

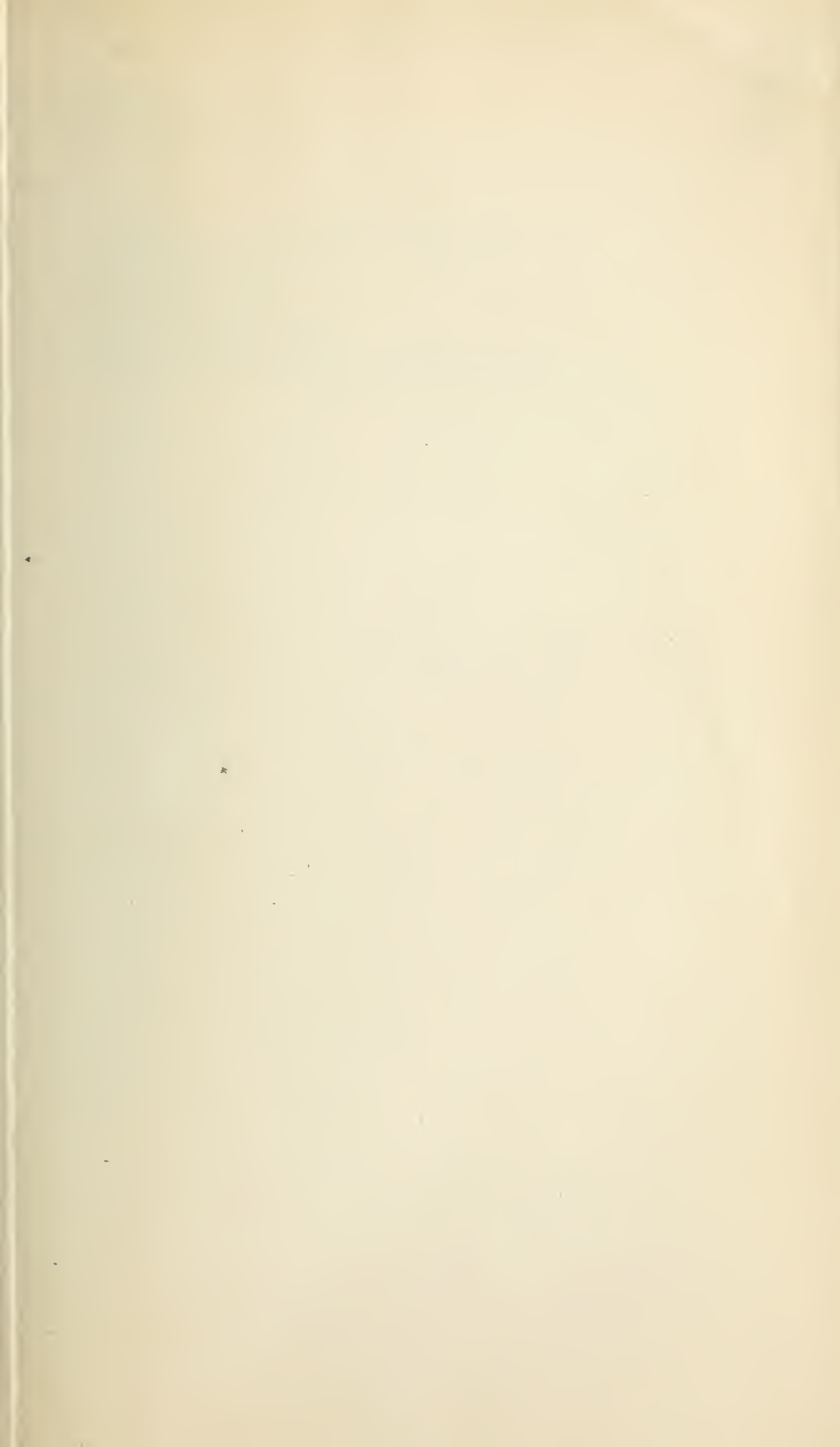
The bedbug.

The roach.

The tick, etc.

Texas fever.

Spotted fever of Bitter Root Valley, Mont.



TRYPANOSOMES—

Inoculate rat with *Trypanosoma Lewisi*.

Dourine.

Surra.

Nagana.

Mal de caderas.

FILARIAL EMBRYOS (“*Filaria sanguinis hominis*”) in the blood.

Filaria Bancrofti Cobbold, 1877.

Filaria diurna Manson, 1891.

Filaria perstans Manson, 1891.

Filaria Demarquayii Manson, 1895.

Dracunculus medinensis (Linnaeus, 1758). [Guinea Worm.]

FERMENTS—

Organized.

Unorganized—enzymes.

A. *Amylolytic*:

Ptyalin.

Diastase.

Amylopsin.

Convert starch and glycogen into sugar.

B. *Proteolytic*:

Pepsin.

Trypsin.

Bacterial peptonizing enzymes.

Convert proteids into peptones.

C. *Steatolytic*—steapsin.

Split fats into fatty acids and glycerin.

D. *Invertin*—in intestinal juice and yeast cells.

Convert cane sugar, maltose, lactose into glucose.

E. *Coagulation*:

Rennet.

Fibrin ferment.

Convert soluble into insoluble ferments.

F. *Alcoholic ferments*:

Saccharomyces.

Converts carbohydrates into alcohol, carbon dioxide, etc.

Fermentation in—

Wine.

Beer.

Vinegar, acetic.

Milk :

Lactic.

Butyric.

Koumys.

Kefir.

IMMUNITY—

Natural.

Artificial.

Active.

Passive.

Acquired.

Serum therapy—

Anti-infectious sera.

Antitoxic sera.

Prophylactic sera.

Curative sera.

Ehrlich's side chain theory.

Phagocytosis.

Preventive vaccines—

Vaccinia (smallpox).

Rabies.

Anthrax.

Symptomatic anthrax.

Swine plague.

Hog cholera.

Rouget du porc.

Plague.

Typhoid fever.

Cholera.

Tetanus.

Diphtheria.

TRANSPORTATION OF DISEASED TISSUES AND BACTERIOLOGIC SPECI-
MENS, ETC., BY MAIL AND EXPRESS.

The Regulations of the Post-Office Department, Order No. 176, March 2, 1900.

Mailing cases.

Methods of sending dry specimens.

Methods of sending moist specimens.

Methods of preparing and forwarding samples for bacteriologic study.

Methods of preparing and forwarding samples for histologic study.



TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 8.

MARCH, 1904.

LABORATORY COURSE

IN

PATHOLOGY AND BACTERIOLOGY

(REVISED EDITION)

BY

M. J. ROSENAU,

DIRECTOR OF HYGIENIC LABORATORY.



WASHINGTON:

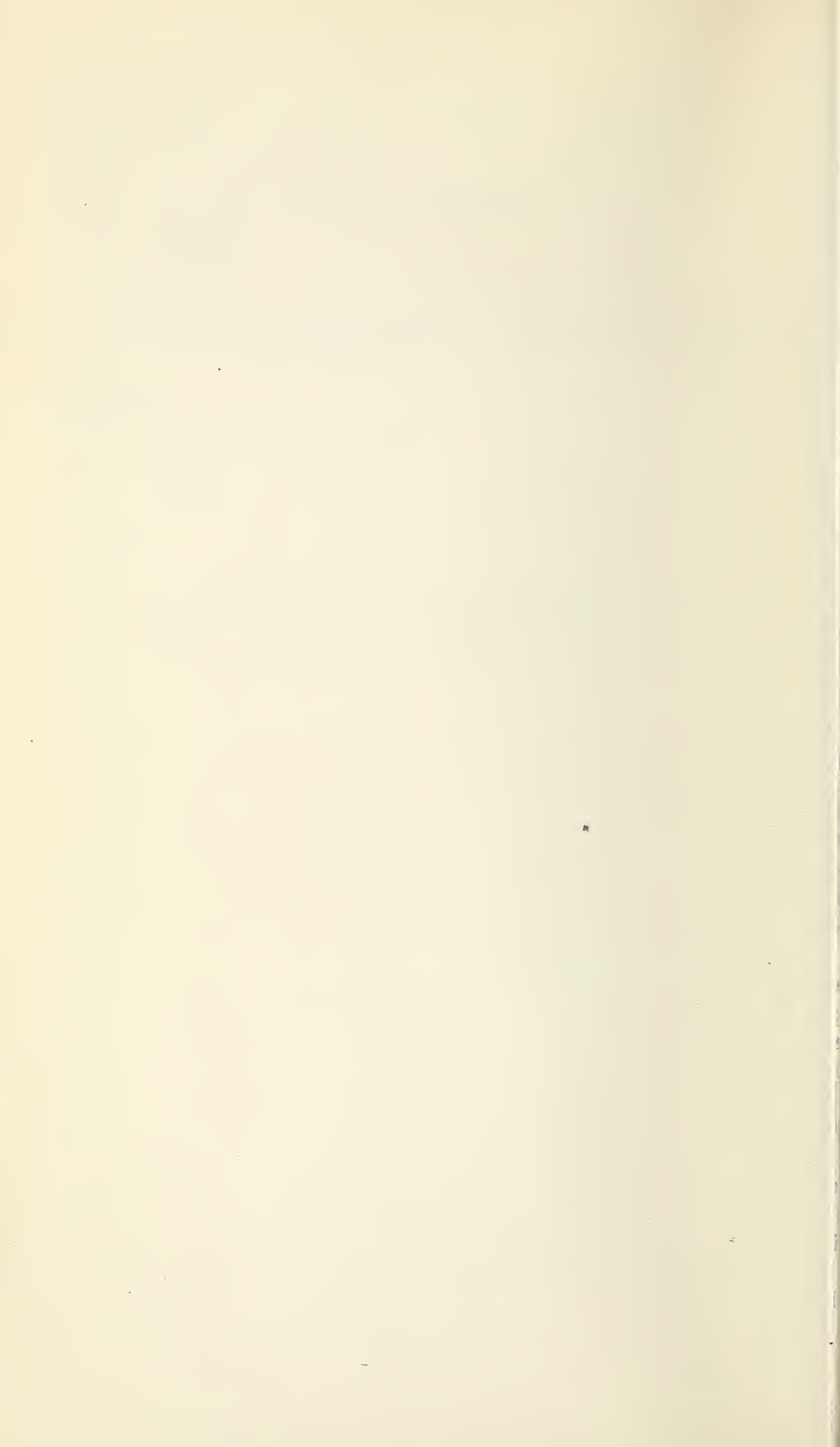
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P R E F A C E .

These pages contain a syllabus of the course in pathology and bacteriology given in this laboratory.

The object of the course is to thoroughly equip officers of the Service in the technique of pathology and bacteriology, in order better to fit them for the hospital, quarantine, epidemic, and public health work of the Service. Officers of the Service who have taken the prescribed course are thoroughly prepared to make a scientific diagnosis of plague, cholera, diphtheria, tetanus, tuberculosis, typhoid fever, anthrax, malaria, and other infectious processes, as well as to carry on original investigations for themselves.

The course as outlined requires one year for its completion. The student-officer is required to work each subject until he has mastered it before going to the next. Such deliberation permits a thoroughness that is not possible when work is hurried or limited to a short period of time.

Special courses.—A special course lasting three months is sometimes given to officers of the Service. This course deals especially with clinical bacteriology and pathology, and consists of the following subjects: Microscopical practice, making of culture media, bacteriological technique, the cutting and staining of pathological specimens, the study of tumors, the diagnosis of diphtheria, tuberculosis, cholera, typhoid fever, anthrax, plague, and malaria; the bacteriological examination of water, and the principles and practice of disinfection.

Another course lasting six months consists of the subjects as above, elaborated in many details. A more thorough study of the saprophytic bacteria is undertaken. A rapid review of histology and general pathology is given, and it also includes a more detailed study of the cultural and biological characteristics of the pathogenic bacteria. This course also includes the technique of blood examinations and the diagnosis of the more common animal parasites.

Modified courses are also given to officers who come to the laboratory well qualified by previous training in both pathology and bacteriology, but who desire to obtain the recent methods and improvements in these subjects, as well as to develop their knowledge in certain branches that have a particular bearing upon the public health.

The exact sequence as given on the following pages is not strictly adhered to, because it is the desire of the director to make the course as practical as circumstances will permit. Therefore, the study of diphtheria is usually left until the fall of the year or winter, when practical experience may be had with the cases which usually occur in the District. The studies upon vaccinia and smallpox are taken up during the winter months, which time is more apt to offer material and cases. Malarial work is of course largely confined to the summer and fall seasons.

In connection with the health department of the District, the work of diagnosing diphtheria from swabs and culture tubes and the administration of antitoxin, both for prophylactic and curative purposes, is carried on by each student-officer. In connection with the studies upon vaccinia, a visit is always made to one of the large vaccine farms, which offers special advantages for observing the process of vaccinating the calves, obtaining the lymph and pulp, glycerinating the virus, and preparing the product for market. In like manner advantage is taken, so far as it is practicable, of the material in hospitals and other institutions situated in the District of Columbia.

It will be noticed that in this edition more attention is given to the subjects of pathologic histology, and a short review in normal histology has also been added. A collection of tumors and pathologic processes has been obtained from various sources and these are cut, stained, mounted, and studied by each student-officer; and these specimens are used to demonstrate the quiz which is conducted by the director of the laboratory. The pathologic histology of all infectious processes upon the lower animals, which are studied in the laboratory, are thoroughly worked up by each student-officer in a systematic manner. Tumors and other specimens are received from time to time from the various hospitals and quarantine stations of the Service, and similar advantage is taken of such material.

In view of the growing importance of animal parasites in the causation of disease, a Division of Zoology has been added to the Hygienic Laboratory and the chief of this division gives each student-officer instruction in the various organisms of this class parasitic to man.

It will also be noticed that considerable importance is given to the subject of disinfection and disinfectants. This arises from the fact that officers of the Service are required not only in their hospital work, but more especially in their quarantine and public health duties, to thoroughly understand the theory and practice of this important subject. Each student-officer is required to master the theory and mechanics of all the important disinfecting apparatus and to make actual tests upon the efficiency and value of each one.

The scientific workers in the laboratory meet one evening each week in order to review the work of the week and to discuss the current



literature. The journals that are received are assigned to each student-officer, and it is his duty to present a critical review of important articles which he reads. We have found this an admirable plan for keeping thoroughly abreast with the large amount of literature constantly appearing, as well as a stimulus to thought and work.

In connection with this literary work each student-officer is encouraged to familiarize himself thoroughly with the use of the library facilities in Washington, in which this city offers unexcelled opportunities.

After the student-officer has been in the laboratory several months and has thoroughly acquainted himself with the necessary technique, opportunity is given him to assist with the work of the laboratory, and every encouragement is offered to work out original problems of his own.

The classes have been small, thus allowing for the individual equation of each student-officer, as well as a maximum of personal attention.



LABORATORY COURSE IN PATHOLOGY AND BACTERIOLOGY.

HYGIENIC LABORATORY,

United States Public Health and Marine-Hospital Service, Washington, D. C.

[By MILTON J. ROSENAU, Passed Assistant Surgeon and Director of the Hygienic Laboratory, United States Public Health and Marine-Hospital Service.]

PREPARATION OF MEDIA—

1. Bouillon:
 - a.* Acid.
 - b.* Neutral.
 - c.* Alkaline.
2. Sugar free bouillon (colon fermentation).
3. Glucose bouillon.
4. Lactose bouillon.
5. Dunham's solution.
6. Gelatin.
7. Agar-agar:
 - a.* Glycerin agar.
 - b.* Litmus agar.
8. Potato.
9. Blood-serum:
 - a.* Fluid.
 - b.* Coagulated.
 - c.* Löffler's blood-serum mixture.
10. Milk.
11. Litmus milk.
12. Aqueous humor of ox eyes.
13. Ascitic fluid.
14. Urine.

STUDIES FOR MICROSCOPIC PRACTICE—

1. Diatoms.
2. Algae.
3. Protozoa.
4. Starch bodies.
5. Vegetable cells.
6. Cotton fibers.
7. Linen fibers.
8. Silk fibers.
9. Feathers.
10. Hair of man and of lower animals.
11. Scales of butterflies and of other insects.
12. Milk.
13. Air bubbles.
14. Oil drops.
15. Vinegar eels (*Anguillula aceti*).
16. Inse casts.
17. Ciliated epithelium.
18. Crystals of sugar, salt, uric acid, cholesterol, hematin, and various chemicals.
19. Decomposing meat.
20. Hay infusion.
21. Surface waters.
22. Opaque objects by reflected light.



POINTS TO BE OBSERVED IN DESCRIBING AN ORGANISM—

1. Its source.
2. Hanging drop. Form, size, grouping and motility.
3. Spores.
4. Staining peculiarities.
5. Flagella.
6. Appearance of colonies on gelatin, agar, blood-serum, potato, etc.
7. Relation to oxygen.
8. Growth on gelatin.
9. Growth on agar.
10. Growth on blood-serum.
11. Growth on potato.
12. Growth on fluid media:
 - a.* Bouillon—
 1. Acid.
 2. Neutral.
 3. Alkaline.
 - b.* Dunham's solution—indol.
13. Milk and litmus milk.
14. Reaction of media best suited to growth.
15. Changes in reaction of media.
16. Gas production:
 - a.* Glucose bouillon.
 - b.* Lactose bouillon.
 - c.* Saccharose bouillon.
 - d.* Mannite bouillon.
 - e.* Glucose agar.
 - f.* Glucose gelatin.
17. Temperature best suited to development.
18. Pathogenicity.
19. Reaction to sera of immune animals, antibodies, precipitins and agglutinins.
20. Toxins.

STUDY OF SAPROPHYTES.

BACTERIA.

Genus *Bacillus* Cohn—

1. *Bacillus subtilis* (Ehrenberg, 1833) Cohn, 1872.
2. *Bacillus vulgaris* (Hauser, 1885) Migula, 1900. [*Proteus vulgaris*.]
3. *Bacillus megaterium* de Barry, 1884.
4. *Bacillus prodigiosus* (Ehrenberg, 1839) Flügge, 1886.

Genus *Pseudomonas* Migula—

5. *Pseudomonas fluorescens* Migula, 1900. [*Bacillus fluorescens liquefaciens* Flügge, 1886.]
6. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872; *Bacillus pyocyaneus* Gessard, 1882.]

Genus *Planosarcina* Migula—

7. *Planosarcina agilis* (Ali-Cohen, 1889) Migula, 1900. [*Micrococcus agilis* Ali-Cohen, 1889.]

Genus *Sarcina* Goodsir—

8. *Sarcina lutea* Schröter, 1886.

Genus *Microspira* Schröter, 1886—

9. *Microspira aquatilis* (Günther, 1892) Migula, 1900. [*Vibrio aquatilis* Günther 1892.]

COMMON MOLDS—

Oidium.

Aspergillus.

Penicillium.

Mucor.

YEASTS—

Beer yeast (*Saccharomyces cerevisiæ*).

Red yeast.

Black yeast.

Saccharomyces (*Torula*) *apiculatus*.

THE SYSTEMATIC POSITION OF BACTERIA.

LOWEST FORMS OF ANIMAL LIFE:

Class Ciliata—*Balantidium coli*.

Class Sporozoa—

Order Coccidiida—The oviform coccidium, *Eimeria stiedæ*.

Order Haemosporidia—Malarial parasites, *Plasmodium*.

Order Sarcosporidia—*Sarcocystis miescheriana*.

Class Mastigophora—

Subclass Flagellata—*Trypanosoma* and *Trichomonas*.

Class Sarcodina—

Subclass Rhizopoda—The ameba of dysentery, *Entamoeba histolytica*.

Class Mycetozoa—

Order Myxomycetes.

This group presents both animal and plant characters.

ALLIED FORMS OF PLANT LIFE:

Algae—

Cyanophyceæ.

Palmaceæ.

Bacteria—

Chlorophyll.

Pleomorphism.

“*Bacterium zophi*.”

Vibrio cholera.

Bacillus pestis.

The bacterial cell:

Its membranes—

Capsule.

Nucleus.

Granules.

Methods of reproduction—

Division.

Spore formation.

CLASSIFICATION OF SCHIZOMYCETES—

1. According to some characteristic feature:

- a.* Chromogenic—production of color.
- b.* Photogenic—production of light.
- c.* Zymogenic—causing fermentation.
- d.* Saprogenic—causing putrefaction.
- e.* Pyogenic—causing formation of pus.

2. According to morphology:

Cocci—

- a.* Micrococci—spherical.
- b.* Diplococci—spheres in pairs.
- c.* Streptococci—spheres in chains.
- d.* Staphylococci—spheres in clusters.
- e.* Tetrads—spheres in double pairs.
- f.* Sarcinae—spheres in bundles.
- h.* Ascococcus—massive colonies with tough, thick, gelatinous envelopes.

Bacilli—

- a.* Leptothrix—filaments of great length.
- b.* Cladothrix—long filaments, pseudo-dichotomy, motile gonidia.
- c.* Streptothrix—branched filaments, mouldy appearance on solid media, aerial hyphae.

Spirilla—

- a.* Vibrio.
- b.* Spirillum.
- c.* Spirocheta. (Probably flagellate animal parasites.)

3. According to motility:

- a.* Nonmotile.
- b.* Motile.
- c.* Brownian motion.

4. According to arrangement of flagella:

- a.* Monotrochal—a single flagellum at the end of the cell.
- b.* Amphitrochal—a single flagellum at each pole.
- c.* Lephotrochal—a cluster of flagella at one pole.
- d.* Peritrochal—a varying number of flagella surrounding the body of the bacterium.

5. According to relation of spores:

- 1. Nonspore bearing.
- 2. Spore bearing—
 - a.* Endospores.
 - b.* Arthrospores.

6. According to relations of host:

- a.* Parasitic; grow and develop in living host.
- b.* Saprophytic; grow and develop upon dead organic matter.
- c.* Pathogenic; induce disease.
- d.* Nonpathogenic; harmless; may be parasitic or saprophytic.

7. According to relations to oxygen:

- a.* Aerobic.
- b.* Anaerobic.

Facultative and obligate.

MAKING PLATES, ETC.

1. Koch's method—glass plates.
2. Esmarch's roll tubes.
3. Slants of agar and gelatin in test tubes.
4. Petri dishes—
 - Streaking on surface with platinum wire.
 - Daubing with swabs on surface.
 - Mixing while fluid.

OBTAINING PURE CULTURES—

1. By plate method on solid media.
2. By dilution in liquid media.
3. By temperature.
4. Inoculation into animals.
5. Negative plates.
6. Special methods.

OBSERVE GROWTH AND MULTIPLICATION OF BACTERIA IN CELL SLIDE—AGAR METHOD:

1. Division of bacilli.
2. Division of cocci.
3. Sporulation.

STUDY OF BROWNIAN MOVEMENT WITH—

India ink and other substances in hanging drop.

COUNTING COLONIES, i. e., number of bacteria.

1. Petri dishes—
 - Aim to have not over 200 colonies on the plate.
 - Count with a lens $\times 5$ D.
 - Wolflügel's counting apparatus.
 - H. W. Jeffer's plate.
2. Esmarch's roll tubes.
3. By dilution in liquid media.

THE PRINCIPLES, CHEMISTRY, AND PRACTICE OF STAINING—

A. Animal stains:

Carmines—

- a.* Acid.
- b.* Alcoholic.
- c.* Alum.
- d.* Borax.

B. Vegetable stains:

Hematoxylin—

- a.* Delafield's hematoxylin.
- b.* Alum hematoxylin.
- c.* Hemalum (Meyer).
- d.* Hematoxylin and eosin.
- e.* Acid hematoxylin.

C. Anilin stains:

1. Acid stains—

- a.* Eosin.
- b.* Acid fuchsin.
- c.* Orange G.
- d.* Soudan III.
- e.* Picric acid.

2. Basic stains—

- a.* Methylene blue (Löffler's solution).
- b.* Thionin.
- c.* Fuchsin (Carbol-fuchsin).
- d.* Gentian violet (Ehrlich's aniline water).
- e.* Methyl violet.
- f.* Bleu d'argent.

D. Special stains—

a. The polychrome blues—

Romanowsky's.

Wright's.

Goldhorn's.

Jenner's.

b. Weigert's fibrin stain.*c.* Mallory's connective tissue stain.

Acid fuchsin.

Phospho-molybdic acid.

Acid fuchsin.

Anilin blue.

E. Gram's method and solution.

F. Decolorization and differentiation:

- a.* Water.
- b.* Alcohol.
- c.* Anilin oil.
- d.* Hydrochlorate of anilin, 2 per cent.
- e.* Acids—organic and mineral.

G. Staining of spores:

Möller's method.

H. Staining of flagella:

- a.* Löffler's method.
- b.* Victoria blue.
- c.* Von Ermengem's method.

PRINCIPLES, CARE, AND USE OF INSTRUMENTS AND APPARATUS IN
THE HYGENIC LABORATORY.

- a.* Centrifuge.
- b.* Crematory.
- c.* Photographic apparatus.
- d.* Cleaning glassware.
- e.* Microscopes.
- f.* Paraffin bath.
- g.* Analytical balances.
- h.* Microtomes.
- i.* Thermostats.
- j.* Thermo-regulators; electric, mercurial, metallic.
- k.* Vacuum pumps.
- l.* Blowpipe.
- m.* Camera lucida.
- n.* Spectroscope.
- o.* X-ray tubes and induction coil.
- p.* Autoclaves.
- q.* Dry wall sterilizer.
- r.* Arnold steam sterilizer.
- s.* Blood serum sterilizer.
- t.* Refrigerating plant, ice box, low temperature incubator, drinking water.
- u.* Sterilization of instruments, glassware, etc.
- v.* Sharpening and care of cutting instruments.

OPTIC PRINCIPLES INVOLVED IN MICROSCOPY—

1. Lenses:
 - a.* Plano-convex.
 - b.* Biconvex.
 - c.* Concave.
 - d.* Biconcave.
 - e.* Doublets.
 - f.* Triplets.
 - g.* Quadruplets.
2. Objectives:
 - a.* Achromatics.
 - b.* Apochromatics.
 - c.* Panchromatics.
3. Oculars:
 - a.* Huyghenian.
 - b.* Compensation.
 - c.* Spectroscopic.
 - d.* Micrometer.
 - e.* Projection.
4. Condensers:
 - a.* Simple—
 1. Biconvex.
 2. Plano-convex.
 - b.* Abbe—
 - Numerical aperture 1.20.
 - Numerical aperture 1.40.
5. Microphotography and projection.
6. Micrometers:
 - a.* Stage.
 - b.* Ocular.
 - c.* Projection.

MEASURING MICROSCOPIC OBJECTS—

1. Eye-piece micrometer.
2. Stage micrometer.
3. Photographic method.

GLASS BLOWING.

1. The blowpipe.
2. Pipettes.
3. Bulbs.
4. Welding.
5. Sealing and opening glass containers.
6. Making test tubes.
7. Making perforated tubes for collodium sacks.

FOLDING PAPER FILTERS.

PREPARING BACTERIOLOGIC FILTERS (PRINCIPLES AND PRACTICE)—

1. Kaolin.
2. Diatomaceous earth.
3. Asbestos.
4. Sand filtration.

PREPARATION AND MOUNTING OF HISTOLOGIC SPECIMENS—

1. Fixation:
 - a.* Acetic-bichlorid of mercury.
 - b.* Müller's fluid.
 - c.* Chrom-acetic acid.
 - d.* Flemming's solution.
 - e.* Formaldehyd.
 - f.* Absolute alcohol.
2. Dehydration:
 - a.* Alcohol 70 per cent (iodin alcohol if HgCl_2).
 - b.* Alcohol 85 per cent.
 - c.* Alcohol 95 per cent.
 - d.* Absolute alcohol, twice.
3. Clearing:
 - a.* Xylol.
 - b.* Chloroform.
 - c.* Turpentine.
 - d.* Oil cloves.
 - e.* Oil bergamot.
 - f.* Oil origanum.
4. Infiltration and imbedding:
 - a.* Paraffin.
 - b.* Celloidin.
5. Sectioning:
 - a.* Single sections.
 - b.* Serial sections.
6. Attaching section to slide:
 - a.* Alcohol 80 per cent.
 - b.* Water.
 - c.* Myer's albumen.
 - d.* Gum Arabic.
 - e.* Collodium and clove oil.
7. Removal of imbedding material:
 - a.* Xylol to remove paraffin.
 - b.* Alcohol and water.
8. Staining:
 - a.* Nuclear staining.
 - b.* Protoplasmic.
 - c.* Regressive.
 - d.* Gram's.
 - e.* Bacterial.
 - f.* Special.
9. Dehydration in absolute alcohol, anilin oil.
10. Clearing:
 - a.* Xylol.
 - b.* Turpentine.
 - c.* Oil origanum.
 - d.* Oil bergamot.
 - e.* Oil cloves.
 - f.* Chloroform.
11. Mounting in xylol balsam, or gum thus.

THE PARAFFIN BATH AND PARAFFIN IMBEDDING—

- A. Fixing.
- B. Dehydration.
- C. Clearing.
- D. Imbedding in paraffin.
- E. Molding block.
- F. Method of numbering, registering, and storing.
- G. Cutting.
- H. Fixing to slide.
- I. Remove paraffin.
- J. Stain.
- K. Dehydrate.
- L. Clear.
- M. Mount in xylol balsam.

CELLOIDIN IMBEDDING—

Fix.

Dehydrate.

Clear.

Prepare a thin celloidin solution containing 4 per cent celloidin in equal parts of absolute alcohol and ether; preserve celloidin solutions carefully from moisture.

Prepare a saturated solution in the same solvent.

Transfer tissue from absolute alcohol to equal parts of alcohol and ether.

From alcohol and ether to thin celloidin.

From thin celloidin to thick celloidin.

From thick celloidin to paper mold on vulcanite block.

Into 70 per cent alcohol for three days to harden.

Cut section with knife flooded with 80 to 85 per cent alcohol.

Preserve in 80 to 85 per cent alcohol.

Stain.

Dehydrate.

Clear.

Mount.

METHOD OF MAKING FROZEN SECTIONS. (Wright.)

1. Place tissue not over 5 mm. thick in 10 per cent formalin for two or more hours.
2. Wash in water.
3. Freeze with CO₂ or ether.
4. Cut frozen sections.
5. Float sections in normal salt solution and then on slide.
6. Cover section with cigarette paper, then with blotter moistened with 95 per cent alcohol. Press. Remove papers.
7. Cover with absolute alcohol thirty seconds. Drain.
8. Flow over the section and adjacent parts of the slide a thin solution of celloidin in equal parts of absolute alcohol and ether. Drain off immediately. The celloidin should form a coating so thin as to be invisible.
9. Flood the slide with 95 per cent alcohol and then immerse in water. This hardens the film.
10. Stain.
11. Dehydrate.
12. Clear with oil origanum.
13. Mount in Canada balsam.

STAINING TISSUES IN SECTIONS.

1. Hemalum and eosin.
2. Delafield's hematoxylin.
3. Eosin and thionin.
4. Carmine stains.
5. Weigert's fibrin stain.
6. Jenner's stain.
7. Van Giesen for connective tissues.
8. Mallory's connective tissue stains.

STAINING BACTERIA IN SECTIONS.

1. Gram's method.
2. Eosin and thionin.
3. Thionin.
4. Methylene blue.

REVIEW OF HISTOLOGY.

Quiz and practical studies.

EPITHELIAL TISSUES.

Classified according to form, arrangement, or special modification:

A. Classification according to form:

1. Squamous—
Simple (pavement).
Stratified.
2. Polyhedral—
Cuboidal.
Sphenoidal.
Glandular.
3. Columnar.

B. Classification according to arrangement:

1. Simple.
2. Transitional.
3. Stratified.

C. Classification according to special modification:

1. Ciliated.
2. Goblet cells.
3. Pigmented.
4. Prickle cells.

The cell—

- Structure.
- Functions.
- Karyokinesis (onion tips).

ENDOTHELIAL CELLS.

1. Serous.
2. Vascular.

SKELETAL TISSUES (CONNECTIVE).

1. Mucous.
2. Reticular.
3. Fibrous—
 - a. Alveolar—
 - Fixed cells.
 - Connective tissue cells.
 - Granular.
 - Plasma.
 - Pigment.
 - Wandering cells.
 - b. Tendons, aponeuroses, etc.
 - c. Elastic.
4. Adipose.
5. Cartilage—
 - a. Hyalin.
 - b. White fibrous.
 - c. Yellow elastic.
6. Bone.

MUSCULAR TISSUES.

1. Striated.
2. Nonstriated.
3. Cardiac.

NERVOUS TISSUES.

1. Nerve cells.
2. Nerve fibers—
 - a. Medulated.
 - b. Nonmedulated.
3. Ganglia.

CIRCULATORY SYSTEM.

1. Arteries.
2. Veins.
3. Capillaries.
4. Heart.
5. Blood—
 - a. Red cells.
 - b. White cells.
 - c. Plaques.

LYMPHATIC SYSTEM AND DUCTLESS GLANDS.

1. Lymph vessels.
2. Lymph glands.
3. Spleen.
4. Thymus.
5. Hemolymph glands.
6. Serous membranes.
7. Synovial membranes.
8. Thyroid.

MUCOUS MEMBRANES AND GLANDS.

1. Mucous membranes.
2. Glands—
 - a. Mucous.
 - b. Serous.

DIGESTIVE TRACT.

1. Sublingual gland.
2. Submaxillary gland.
3. Tongue (papillæ).
4. Tonsils.
5. Esophagus.
6. Stomach (glands).
7. Intestines—
 - a.* Villi.
 - b.* Glands.
 - a.* Lieberkühn's.
 - b.* Brunner's.
 - c.* Solitary.
 - d.* Peyer's patches.
8. Liver.
9. Gall bladder.
10. Parotid.
11. Pancreas.

URINARY ORGANS.

1. Kidney.
2. Ureter.
3. Bladder.
4. Urethra.

MALE SEXUAL ORGANS.

1. Testicle.
2. Prostate.
3. Penis.

FEMALE SEXUAL ORGANS.

1. Ovary.
2. Uterus.
3. Vagina.
4. Mammary gland.

RESPIRATORY ORGANS.

1. Trachea.
2. Bronchi.
3. Lungs.
4. Pleura.

SKIN AND APPENDAGES.

1. Skin.
2. Hair.
3. Sebaceous glands.
4. Sweat glands.

CENTRAL NERVOUS SYSTEM.

1. Spinal cord.
2. Cerebrum.
3. Medulla.
4. Cerebellum.

Cut, stain, mount, and study sections of the following normal tissues:

Skin.

Tongue.

Tonsil.

Esophagus—

Upper.

Lower.

Stomach—

Pyloric.

Cardiac.

Intestine—

Upper duodenum.

Lower ileum.

Large.

Liver.

Gall bladder.

Parotid.

Sublingual.

Submaxillary.

Pancreas.

Spleen.

Kidney.

Ureter.

Bladder.

Penis.

Testicle.

Prostate.

Ovary.

Uterus.

Vagina.

Mammary gland.

Voluntary muscle.

Tendon.

Heart.

Artery and vein.

Lymph gland.

Epiglottis.

Trachea.

Lung.

Thyroid.

Intravertebral disc.

Sciatic nerve.

Spinal cord.

Cerebellum.

Cerebrum.

Medulla.

Embryo.

Umbilical cord.

REVIEW OF PATHOLOGY.

Quiz and practical studies.

GENERAL PATHOLOGY:

Inflammation—

Causes.

Study phenomenon in omentum of frog, etc.

Changes in the vessels.

Exudation.

Changes in the tissues.

Proliferation.

Special forms of inflammation—

Edematous or serous inflammation.

Fibrinous inflammation.

Diphtheritic inflammation. (Coagulation necrosis.)

Croupous inflammation.

Suppurative (abscess) inflammation.

Catarrhal inflammation.

Parenchymatous inflammation.

Productive (interstitial) inflammation.

Hemorrhagic inflammation.

Necrotic or gangrenous inflammation.

Resolution after inflammation.

Regeneration.

Degenerations and infiltrations:

Atrophy.

Cloudy swelling.

Fatty infiltration.

The albuminoid degenerations:

Hyalin.

Mucoid.

Colloid.

Amyloid.

Glycogenic infiltration.

Dropsical infiltration.

Calcification.

Ossification.

Uratic infiltration.

Pigmentation.

Necrosis.

Coagulation necrosis.

Liquefaction necrosis.

Caseation.

Fat necrosis.

Gangrene.

STUDY OF TUMORS.

CAUSE:

- External irritation. (Virchow.)
- Defective development—embryonal rests. (Cohnheim.)
- Infectious character—parasitic theory. Cell inclusions.
- Constitutional discrasia.
- Loss of compensation between cellular destruction and proliferation.

CLASSIFICATION:

- Histogenetic—based on structure.
- Embryologic—based on embryologic derivation of tissue forming the tumor.
- Clinical—benign, malignant.

METASTASIS.

Cut sections and study the following, which will be found in the laboratory collection:

1. Myoma.
2. Neuroma.
3. Angioma:
 - a. Simple.
 - b. Cavernous.
4. Lymphangioma:
 - a. Simple.
 - b. Cavernous.
5. Fibroma.
 - Soft fibroma.
 - Hard fibroma.
 - Intracanalicular fibroma—of breast.
6. Myxoma.
7. Lipoma.
8. Chondroma.
9. Osteoma.
10. Glioma.
11. Sarcoma:
 - a. Round celled.
 - b. Lympho-sarcoma.
 - c. Alveolar.
 - d. Spindle celled.
 - e. Melano-sarcoma.
 - f. Osteoid.
 - g. Psammoma.
 - h. Myeloid.
 - i. Angiosarcoma.
 - j. Endothelioma.
12. Papilloma.
13. Adenoma.
14. Carcinoma:
 - a. Acinous.
 - b. Epithelioma.
15. Cystoma.
16. Hypernephroma.

SPECIAL PATHOLOGY.

A brief review of the more important lesions.

Put, mount, stain, and study the following specimens, which will be found in the laboratory collection:

Liver—

- Parenchymatous degeneration.
- Hyalin degeneration.
- Fatty degeneration.
- Spleno-myelogenous leukemia.
- Amebic abscess wall.
- Yellow fever liver.
- Malarial liver.

Kidney—

- Parenchymatous degeneration.
- Amyloid kidney.
- Infected infarct.
- Miliary tuberculosis.
- Large red kidney.
- Large white kidney.
- Granular and contracted kidney.
- Spleno-myelogenous leukemia.
- Hydronephrosis.
- Yellow fever kidney.
- Malarial kidney.

Lungs—

- Chronic passive congestion.
- Croupous pneumonia, red hepatization.
- Croupous pneumonia, gray hepatization.
- Catarrhal pneumonia.
- Bronchiectasis.
- Emphysema.
- Unresolved pneumonia.
- Gumma of lung.

Gastro-intestinal canal—

- Acute catarrhal gastritis.
- Stomach—yellow fever.
- Acute colitis.
- Acute enteritis.
- Follicular enteritis.
- Diphtheritic enteritis.
- Infiltrated Peyer's patches.
- Typhoid ulcer—ileum.
- Cicatrized Peyer's patches.

Circulatory system—

- Arteriosclerosis.
- Aneurismal wall, with thrombus.
- Vessels in yellow fever.

Cut, mount, stain, and study the following specimens, which will be found in the laboratory collection—Continued.

Lymphatic system—

Hyalin degeneration—lymph gland.

Tuberculosis—adenitis.

Primary lymphatic sarcoma.

Lymphatic leukemia.

Typhoid spleen.

Amyloid spleen.

Hodgkins's disease—enlarged glands.

Mucous membranes—

Diphtheritic inflammation, trachea.

Serous surfaces—

Acute fibrinous peritonitis.

Acute pericarditis.

Chronic endocarditis.

Brain and spinal cord—

Spinal cord—cerebrospinal meningitis, diplococcal.

Spinal cord—cerebrospinal meningitis, epidemic.

External pachymeningitis.

Acute meningitis.

Gumma of meninges.

Tuberculous meningitis.

Posterior sclerosis.

Syphilis—

Gumma.

Tertiary lesions—larynx.

Leprosy—

Nerve lesion.

Skin leproma.

Actinomycosis—

Lung.

Tongue.

Other pathologic processes are studied from time to time as they are received for diagnosis.

ANIMAL INOCULATIONS—

1. Subcutaneous method:
 - a.* Liquids.
 - b.* Solids.
2. Intravenous method.
3. Inoculation into lymphatic system.
4. Inoculation into serous cavities:
 - a.* Peritoneum.
 - b.* Pleural cavity.
 - c.* Subdural space.
5. Inoculation into anterior chamber of eye.

OBSERVATION OF ANIMALS AFTER INOCULATION—

1. Temperature.
2. Loss of weight.
3. Peculiar position in cage.
4. Loss of appetite.
5. Condition of coat.
6. Condition of secretions, etc.
7. Pain.
8. Nervous symptoms, etc.

POST-MORTEM EXAMINATION OF ANIMALS—

1. Cultures from the organs.
2. Cultures from the fluids.
3. Preparation and staining of tissues.

INCINERATION OF DEAD ANIMALS AFTER EXAMINATION—

MAKING COLLODIUM SACKS—

- Paris method.
- Gelatin capsule method.
- With glass support and gelatin (method of Grubbs & Francis).

BACILLUS COLI Migula, 1900. [*Bacterium coli commune* Escherich.]

- A. Distribution.
- B. Isolate from sewage.
- C. Characteristics:
 - 1. Motility.
 - 2. Gram's method.
 - 3. Nonliquefaction of gelatin.
 - 4. Indol.
 - 5. Gas production.
 - 6. Colonies, etc.
 - 7. Production of acid.
- D. Pathogenesis.
- E. Varieties.
- F. Preparation of its toxin.
- G. Pathogenicity.
- H. Study of blood and organs of infected animals.
- I. Significance in drinking water:
 - 1. Isolation from drinking water.
 - 2. Counting number of colon organisms in a cubic centimeter of water.
- J. Viability.



BACILLUS ANTHRACIS Koch, 1876.

- A. History.
- B. Distribution.
- C. Morphology.
 - 1. Involution forms.
 - 2. Asporogenic form.
- D. Staining.
- E. Cultural characteristics.
- F. Spores:
 - 1. Formation—
 - Relative to oxygen.
 - Relative to nutrient media.
 - Relative to temperature.
 - 2. Development into bacteria.
- G. Pathogenesis (mouse, guinea pig, and rabbit).
 - a.* Pneumonic.
 - b.* Hemorrhagic.
 - c.* Local.
- H. Microscopic examination of liver, blood, spleen, lungs, and other organs.
- I. Vaccination:
 - First vaccine—kills mice, not guinea pigs or rabbits.
 - Second vaccine—kills mice and guinea pigs, not rabbits.
 - Third vaccine—kills mice and guinea pigs and some rabbits.
- J. Disposal of anthrax carcasses:
 - a.* Burial, isolated districts.
 - b.* Burning.
 - c.* Sulphuric acid.
 - d.* Boiling.
- K. Human anthrax:
 - a.* Malignant pustule.
 - b.* Wool sorter's disease.
 - c.* Intestinal form.
- L. Viability.
- M. Disinfection for anthrax.

BACTERIUM DIPHTHERIÆ (Löffler) Migula. [*Klebs-Löffler Bacillus.*]

- A. History.
- B. Morphology—relation to clinical types.
- C. Staining:
 - 1. Löffler.
 - 2. Neisser.
 - 3. Roux.
 - 4. Weigert's fibrin method for tissues.
 - 5. Gram's stain.
- D. Biologic characteristics.
- E. Pathogenesis:
 - 1. Guinea pig.
 - 2. Rabbit.
 - 3. Kitten.
- F. Toxin production.
- G. Antitoxin production:
 - 1. Standardization.
 - 2. Prophylactic use.
 - 3. Curative use.
- H. Obtain tubes and swabs from the health department for diagnosis.
- I. Pseudo-diphtheria bacillus.
- K. Xerosis bacillus.
- L. Viability.
- M. Disinfection for diphtheria.
 - The disease—
 - Symptoms.
 - Diagnosis.
 - Treatment.
 - Prophylaxis.

DUCREY'S BACILLUS—Soft chancre—

Obtain in pure culture.

Diagnosis from smear preparations.

Study morphology—

Cultural characteristics.

Relation to lesions.

MICROCOCCUS MELITENSIS Bruce, 1893. (Malta fever.)

Morphology.

Motility.

Cultural characteristics.

Occurrence in spleen.

Serum diagnosis—agglutinins.

The disease—

Its geographical distribution.

Diagnosis.

Symptoms.

Treatment.

Prophylaxis.

BACTERIA IN INFLAMMATION AND SUPPURATION—

Study:

- A. *Micrococcus aureus* (Rosenbach, 1884) Migula, 1900. [*Staphylococcus pyogenes aureus* Rosenbach, 1884.]
- B. *M. pyogenes* (Rosenbach, 1884) Migula, 1900. [*S. pyogenes albus* Rosenbach, 1884.]
- C. *M. citreus* (Passet, 1885) Migula, 1900. [*S. pyogenes citreus* Passet, 1885.]
- D. "*Staphylococcus epidermidis albus* Welch."
- E. *M. gonorrhæe* (Bumm, 1885) Flügge, 1886.
- F. *Streptococcus pyogenes* Rosenbach, 1884.
- G. "*Streptococcus erysipelatus* Rosenbach, 1884.
- H. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872; *Bacillus pyocyaneus* Gessard, 1882.]

Collect pus and inflammatory exudates from various sources, plate out and study bacteria found.

Study pus in smears on slides.

Also suppurating processes induced by—

- The typhoid bacillus.
- Colon bacillus.
- Pneumococcus.
- Tubercle bacillus, etc.

THE TYPHOID GROUP—

Compare:

- A. *Bacillus typhosus* Migula, 1900.
 - B. *Bacillus dysenteriae* Shiga, 1898 [not Migula, 1900].
 - C. *Bacillus enteritidis* Gaertner, 1888.
 - D. Paracolon bacillus.
 - E. Paratyphoid bacillus.
 - F. *Bacillus murium* Migula, 1900. [*Bacillus typhi murium*, Löffler, 1893.]
 - G. Bacillus of Danyz's virus.
 - H. *Bacillus psitticosis*.
 - I. *Bacillus icteroides* Sanarelli, 1897.
 - J. *Bacillus acidi lactici*.
 - K. *Bacillus coli* Migula, 1900. [*Bacterium coli commune*, Escherich.]
1. Methods of introduction into the system.
 2. Channels of elimination.
 3. Viability.
 4. Disinfection.
 5. Relation to water supply.
 6. Relation to flies and other insects.
 7. Gruber-Widal reaction with live and dead cultures:
 - a. With hanging drop.
 - b. In pipettes and small test tubes.
 8. Wright's preventive inoculation.
 9. Diagnosis of typhoid fever in early stages.
 - Diazo reaction.
 10. Pathologic lesions.
 11. The management of epidemics.
 12. Prophylaxis.

THE TUBERCLE GROUP—

A. *Bacillus tuberculosis* Koch, 1882:

1. Human. Isolate in pure culture from sputum, or lesion.
2. Bovine.
3. Avian.

Morphology.

Biological characters.

Pathogenesis.

Diagnosis.

Tuberculin—

Production.

Uses.

1. Diagnostic.
2. Therapeutic.

B. Acid proof organisms, resembling *B. tuberculosis*:

1. Möller's grass bacillus. Isolate from timothy.
2. Rabinowitsch's butter bacillus.
3. Karlinsky's nasal secretion organism.
4. Smegma bacillus.

C. *Bacillus lepræ* Hansen.

Cut sections leproma.

D. *Bacillus mallei* (Löffler, 1886) Migula, 1900:

Mallein.

Strauss's method in male guinea pig.

E. Actinomycosis.

F. Mycetoma, madura foot.

G. Pathogenic streptothrices.

THE VIBRIO GROUP—

- A. *Microspira comma* Schröter, 1886. [Koch's comma bacillus of Asiatic cholera.]
- B. *Microspira metshnikovi* (Gamaleia, 1888) Migula, 1900.
- C. *Microspira finkleri* Schröter.
- D. *Vibrio Denecke*.
- E. *Microspira aquatilis* (Günther, 1892) Migula, 1900.

Isolation.

Diagnosis.

Pfeiffer's phenomenon.

Widal reaction.

Immunity.

Haffkine's prophylactic.

Viability.

Disinfection for cholera.

Methods of introduction into system.

Channels of elimination.

Relation to water supply.

Relation to flies and other insects.

Quarantine regulations relating to cholera.

The management of isolated cases.

The control of epidemics.

Prophylaxis.

BACILLUS PESTIS Kitasato & Yersin, 1894.

A. Study—morphology, staining, and cultural characteristics:

Viability.

Channels of entrance.

Channels of elimination.

Pathogenicity—guinea pigs, rabbits, rats, mice.

Diagnosis—Kolle's method: "

Yersin serum—

Method of manufacture and standardization.

Prophylactic use.

Curative use.

Haffkine prophylactic—

Methods of manufacture.

Uses.

B. Disinfection.

C. Quarantine.

D. Relation of rats and other mammals to spread of plague.

E. Relation of fleas, flies, and insects to spread of plague.

The disease—

Period of incubation.

Symptoms.

Diagnosis.

Varieties.

Lesions.

Treatment.

Management of epidemics.

Prophylaxis.

THE SEPTICEMIC GROUP—

- A. Mouse septicemia.
- B. Rouget du porc.
- C. Chicken cholera.
- D. Swine plague.
- E. Ferret, duck, buffalo, and other plagues.
- F. *Bacillus pestis* Kitasato & Yersin, 1894.
- G. *Bacterium pneumoniae* (Weichselbaum, 1886) Migula, 1900. [*Diplococcus pneumoniae* Weichselbaum, 1886.]
- H. *Bacterium pneumonicum* Migula, 1900. [*Pneumonicoccus* Friedländer, 1882.]
- I. *Bacterium influenzae* Lehmann & Neumann, 1896.

ANAEROBIC TECHNIQUE—

- A. Kipps's hydrogen apparatus:
 - 1. Mechanics.
 - 2. Chemicals.
- B. Simple hydrogen generators.
- C. Solutions for purifying gas:
 - 1. Silver nitrate for chlorine.
 - 2. Potassium permanganate for oxygen.
 - 3. Lead acetate for sulphides.
- D. Media best suited for anaërobes:
 - 1. Freshly prepared media.
 - 2. Glucose media.
- E. Special methods:
 - 1. Fraenkel's method.
 - 2. Esmarch's method.
 - 3. Liborius's method.
 - 4. Buchner's method.
 - 5. Novy's jars.
 - 6. Wright's method.
 - 7. Pasteur Institute method.
 - 8. Vignal's tubes.
 - 9. Full tubes of bouillon.
 - 10. Deep stabs.
 - 11. Oil method.
 - 12. Smith's apparatus.

ANAEROBES—

A. *Bacillus tetani*, its toxin and antitoxin.

B. *Bacillus œdematis* of malignant edema.

C. *Bacillus Chauvæi* Arloing, Cornevin & Thomas, of symptomatic anthrax:

Vaccine—

1. Manufacture.

2. Use.

D. *Bacillus welchii* Migula, 1900. [*Bacillus aërogenes capsulatus* Welch & Nuttall, 1892.]

Disinfection for above.

PATHOGENIC MOLDS—

*Ringworm:**Microsporon Audouinii* (Gruby).*Trichophyton*—1. *T. endothrix*.2. *T. ectothrix*.

Hair lesions.

Pure cultures.

*Favus:**Achorion Schoenleinii* Remak.

In the hair and scale.

In pure culture.

*Thrush:**Oidium albicans* (see *Saccharomyces albicans*).

YEASTS—

Saccharomyces cerevisiæ Meyen (beer yeast) in pure cultures:

Effect on saccharine media.

Budding.

Red yeast—*S. glutinis*.

Black yeast—*S. niger*.

Saccharomyces albicans (Robin) Reess, 1877 [*Oidium albicans* Robin] thrush, in pure culture.

Pathogenic yeasts.

EXAMINATION OF URINE—

Note:

1. Odor.
2. Color.
3. Deposit. Filtration of specimen.
4. Specific gravity.
5. Reaction.
6. Quantity in twenty-four hours.
7. Albumen—

Tests for albumen; quantitative and qualitative:

- a.* Heat (phosphates).
- b.* Nitric acid, in test tube and in glass.
- c.* Ferrocyanid potash; acetic acid.
- d.* Picric acid, Esbach's albumenometer.

8. Sugar—

Test for sugar:

- a.* Fehling's solution.
- b.* Bismuth.
- c.* Quantitative estimation with—
Yeast fermentation.
Fehling's solution.
Polariscope.

9. Estimation of urea.
10. Bile, test for.
11. Microscopic examination of sediment for bacteria, casts, crystals, blood, etc.,
Schistosoma hematobium (Bilharz, 1852) Weinland, 1858.
12. Test for chlorids.
13. Diazo reaction.
14. Bacteriologic examination of urine.
15. Disinfection of urine.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF FECES—

1. Microscopic:

a. Normal constituents.

b. Intestinal parasites and their eggs—

Ascaris lumbricoides (Linnæus, 1758).*Oxyuris vermicularis* (Linnæus, 1758) Bremser, 1819.*Trichuris trichiura* (Linnæus, 1771).*Agchylostoma duodenale* (Dubini, 1843).*Uncinaria* (*Necator*) *americana* Stiles, 1902.*Tænia saginata* Gœze, 1782.*Tænia solium* Linnæus, 1758.*Hymenolepis nana* (Siebold, 18—).*Dibothriocephalus latus* (Linnæus, 1758.)*Entamæba coli* (Lœsch, 1875)=*Amæba coli* [nonpathogenic].*Entamæba histolytica* Schaudinn, 1902=*Amæba* of dysentery [pathogenic].

2. Bacteriologic:

a. Normal flora.

b. Pathogenic—

Bacillus dysenteria.*Bacillus tuberculosis*.*Bacillus typhosus*.*Microspira comma*.

3. Disinfection of feces.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF—

Sputum, nasal and other mucous secretions:

 Tubercle bacilli.

 Pneumococci.

 Influenza bacterium.

Disinfection of sputum.

BLOOD.

Technique of:

1. Collecting blood for microscopic examination.
2. Collecting blood for bacteriologic examination—
 - a.* Small amount.
 - b.* Large quantities.
 Of man.
 Of other mammals.
 Of birds.
 Of reptiles.
3. Observe circulation of blood under microscope.
 Mesentery of frog.
4. Estimate alkalinity.
5. Estimate specific gravity of blood with mixture of chloroform and benzol.
6. Examine fresh blood in hanging drop.
7. Examine fresh blood between slide and cover slip.
8. Study of blood films:
 - a.* Method with cover glass.
 - b.* Method with special rod.
 - c.* Method with cigarette paper.
9. Fixation of blood films:
 - a.* Heat, 150° C. for about five minutes.
 - b.* Alcohol and ether.
 - c.* Formalin.
 - d.* Formaldehyd.
 - e.* Acetic acid bichlorid.
 - f.* Wood alcohol.
10. Staining:
 - a.* Ehrlich-Biondi-tri-acid stain.
 - b.* Jenner's stain.
 - c.* Romanowsky's stain.
 - d.* Goldhorn's fluid.
 - e.* Eosin and methylene blue.
 - f.* Thionin and eosin.
 - g.* Wright's.
 - h.* Hematoxylin and eosin.
11. Counting red corpuscles.
 Thoma-Zeiss hemocytometer.
12. Estimating the number of leucocytes:
 - a.* With the Thoma-Zeiss hemocytometer.
 - b.* Comparative count of—
 - a.* Small lymphocytes.
 - b.* Large lymphocytes and transitional forms.
 - c.* Polymorphonuclear neutrophiles.
 - d.* Eosinophiles.
 - e.* Mast cells.
13. Estimating the amount of hemoglobin:
 With—
 - a.* Dare's hemoglobinometer.
 - b.* Oliver's hemoglobinometer.
 - c.* Gower's hemoglobinometer.

BACTERIA IN THE AIR—

1. Number:

Sewer air.

Expired air.

Sea air.

City air.

Country air.

Air in houses, hospitals, etc.—

Relation to the sun.

Relation to habitation.

Relation to winds and traffic.

Relation to altitude.

Pasteur's method.

Miquel's method.

Aeroscopes.

Frankland's method.

Petri's method.

Hesse's method.

Strauss's method.

2. Varieties.

3. Estimation of ammonia in the air.

4. Estimation of carbon dioxid in the air.

BACTERIA IN WATER—

1. Collecting samples.
2. Count number per c. c.:
 - a. In tap water.
 - b. In river water.
 - c. In spring water.
 - d. In rain water.
 - e. In ice.
3. Species:
 - Coli communis.
 - Typhoid.
 - Cholera.
4. Organisms in water:
 - a. Selection.
 - b. Isolation.
 - c. Numbers.
5. Estimation of organic matter—
 - a. Free ammonia.
 - b. Albuminoid ammonia.
6. Estimation of nitrates and nitrites.
7. Estimation of chlorids.
8. Purification of water:
 - Heat.
 - Filters—
 - a. Household filters.
 - b. Field and camp filters.
 - c. Sand filters—
 - Alum.
 - Hyperoxid of chlorin.
 - Ozone.
 - Permanganate of potash.

BACTERIA IN SOIL—

1. Number.
 2. Varieties.
- Nitrification.

PURIFICATION AND DISPOSAL OF SEWAGE.

1. Dilution.

This method is allowable for cities situated on the sea or on rivers whose flow is very large as compared with the sewage (100:1) and the unfiltered water of which is not used as a water supply.

2. Sewage farming.

3. Chemical precipitation.

4. Intermittent filtration.

Needs sandy soil.

5. Septic tank.

6. Contact bed treatment.

7. Continuous filtration:

a. Scott-Moncrieff system.

b. The Ducat.

c. Whitaker & Bryant.

d. F. Stoddart.

e. G. Salford.

MILK—

1. Histology.
2. Bacteria in milk:
 - Number.
 - Origin.
 - Varieties.
3. Fermentation—
 - Lactic acid.
 - Butyric acid.
 - Sweet curdling.
 - Abnormal.
4. Pasteurization:
 - Theory and practical uses.
 - Changes produced in milk.
5. Count and study colonies in fresh milk and milk bought in open market.
 - Pasteurize, then count and study colonies.
6. Sterilization of milk by heat; practical application and changes produced in milk.
7. Milk preservatives.
8. Diseases conveyed by milk.

PRINCIPLES AND PRACTICE OF DISINFECTION AND STERILIZATION.

Physical agents.

1. Sunlight—

Expose spores of anthrax, subtilis, etc.

Expose nonspore-bearing organisms as typhoid, diphtheria, etc., to the sunlight in thin films and for penetration. Note intensity of the light, temperature, dryness, and other conditions. Plant in bouillon.

2. Electricity—

Expose slips and cultures to various currents.

Expose to electric light.

Expose to X-rays.

3. Dryness and dry heat—

Expose slips, or threads with spore-bearing and nonspore-bearing organisms to:

a. Dryness at room conditions.

b. Dry in incubator at 37° C.

c. Dry over sulphuric acid.

d. Dry heat at 5° C.,

15° C.,

37° C.,

for various times.

e. Expose paper slips saturated with—

a. Decomposing urine,

b. Sewage,

c. Garden earth,

to 150° C. for one hour to test power of sterilization of this temperature.

Hot-air sterilizer.

NOTE.—Plant in bouillon and keep one week for growth.

4. Moist heat—hot and boiling water and steam:

Expose typhoid, diphtheria, and other nonspore-bearing organisms to 50°, 60°, 70°, 80°, and 100° C. moist heat for variable times; plant in bouillon.

Expose anthrax, subtilis, and other spores to 70°, 80°, 90°, and 100° C. moist heat for variable times; plant in bouillon.

5. Theory, mechanics, and practical application of steam in—

a. Koch steamer.

b. Arnold steam sterilizer.

c. Autoclave (steam under pressure).

d. Steam chamber (steam under pressure).

e. Superheated steam.

Make practical tests in each.



TESTING ANTISEPTICS—

If the substance belongs to the Cresol group, prepare 1 per cent, 2 per cent, etc., solutions of the substance in a set of bouillon tubes.

If the substance belongs to the metallic salts, make $\frac{1}{5000}$, $\frac{1}{3000}$, $\frac{1}{2000}$, $\frac{1}{1000}$, $\frac{1}{250}$, etc., solutions in a set of bouillon tubes.

Inoculate with:

- a. Decomposing urine.
- b. Sewage.
- c. Garden earth.
- d. Typhoid.
- e. Pus cocci.
- f. Anthrax spores, etc.

Grow at favorable temperature and record each day the appearance of growth, and make report on the following plan:

Name of substance.—Strength of solution.—Temperature.	Days.							
	1.	2.	3.	4.	5.	6.	7.	8.
Sewage	—	—	—					
Garden earth	—	+	+					
Decomposing urine	+	+	+					

— means no growth; + means growth.

TESTING GERMICIDES—

Saturate silk threads,
paper slips,
glass slips, etc., with
subtilis spores,
anthrax spores,
typhoid bacillus,
diphtheria bacillus,
pus cocci, etc.

Expose to the solutions of germicide in varying dilutions and for varying times
at constant temperature, and at the same dilution at varying temperatures.

Wash or neutralize the germicide.

Plant in bouillon.

Incubate.

Report growth upon similar table to antiseptics.

Test the following upon the above plan—

- A. Bichlorid of mercury.
- B. Carbolic acid and tricesol.
- C. Lime.
- D. Copper and iron sulphates.
- E. Formalin.



GASEOUS DISINFECTION—

A. Formaldehyd:

- a.* Chemistry and physics.
- b.* Sheet method.
- c.* Generator—
 - 1. Kuhn lamp.
- d.* Regenerators without pressure—
 - 1. Trenner-Lee.
 - 2. Lentz.
- e.* Regenerators with pressure—
 - 1. Autoclave.
- f.* Heating paraform.
- g.* Spraying.

B. Sulphur dioxid (SO_2):

- a.* Liquid sulphur dioxid.
- b.* Burning sulphur, pot method.
- c.* Sulphur furnace.

C. Hydrocyanic acid gas.

D. Chlorin gas.

E. Ozone and oxygen.

INSECTICIDES—

- A. Tobacco smoke.
- B. Pyrethrum.
- C. Hydrocyanic acid.
- D. Formaldehyd gas.
- E. Sulphur dioxid.
- F. Coal oil.
- G. Arsenical compounds.
- H. Phosphorus.
- I. Cobalt salts.
- J. Carbon bisulphid.

Ants.

Mosquitoes.

Roaches.

Fleas.

Flies, etc.

1. THE DISINFECTION OF HOUSES, SHIPS, AND OBJECTS.
2. THE DISINFECTION AND PROPHYLAXIS AGAINST THE COMMUNICABLE DISEASES, ESPECIALLY.
 - a.* Yellow fever.
 - b.* Cholera.
 - c.* Plague.
 - d.* Smallpox.
 - e.* Tuberculosis.
 - f.* Typhoid fever.
 - g.* Malaria.
 - h.* Dysentery.
 - i.* Diphtheria.
 - j.* Pneumonia.
 - k.* Leprosy.
 - l.* Anthrax.
 - m.* Tetanus.
 - n.* Dengue.
 - o.* Relapsing fever.
 - p.* Typhus fever.
 - q.* Scarlet fever.
 - r.* Measles.

Including channels of introduction and elimination.

Viability.

Disinfection.

Prophylaxis.

Management of epidemics.

Quarantine.

RABIES—

Study in dogs.

Study in rabbits.

Prepare spinal cords for preventive inoculation.

Principles and methods of Pasteur treatment.

VACCINATION (*vaccinia*)—

- A. History.
- B. Methods of production:
 - Vaccine lymph.
 - Vaccine pulp—
 - Dry points.
 - Glycerinated pulp.
- C. Technique of vaccination.
- D. Immunity produced.
- E. Bacteriologic and microscopic study of vaccine matter.
- F. Bodies in epithelial cells—cornea of rabbits.

The examination of vaccine under the law of July 1, 1902.

Note the name of maker, license number, kind of virus, date of return, etc.

a. Count the number of colonies per point.

Immerse the point in 1 cc. physiological salt solution 15 minutes to soften the vaccine matter.

Scrape well and agitate to break up clumps, etc.

Plate the emulsion thus obtained in three Petri dishes as follows:

5 drops in first.

10 drops in second.

Remainder in third.

Then plant in melted agar, incubate at 37° C. for three days, then room temperature for two days.

Count number and determine character of colonies.

b. Count number of colonies per capillary tube.

Wash contents of tube into 1 cc. sterile water.

Agitate to break up clumps.

Plate in agar as above.

c. Plant the entire contents of 10 tubes or 10 points in 100 cc. of freshly prepared and recently boiled glucose bouillon.

Incubate at 37° C.

At the end of 48 hours inoculate upper growth into peritoneal cavity of a rabbit, using 1 cc. per 1,000 gm. of rabbit.

If rabbit sickens or dies determine cause of the infection.

At the end of seven days filter the bouillon growth through porcelain and inoculate 0.2 cc. of the filtrate subcutaneously into a mouse for tetanus toxin.

MALARIA AND MOSQUITOES—

1. Diagnosis.
2. Blood technique.
3. Relation to mosquito:
 - a. Tertian.
 - b. Quartan.
 - c. Estivo-autumnal.

Mosquitoes—

Anatomy—dissections. fresh specimens.

Sections in paraffin.

Development and raising.

Classification.

Diagnosis, especially:

- a. *Culex*.
 - b. *Anopheles*.
 - c. *Stegomyia*.
- A. Malaria—the *Anopheles*:
- Study *Anopheles* one each day after biting a malarial case.
- Tertian.
- Quartan.
- Estivo-autumnal.
- B. Yellow fever and the mosquito.
- C. Filaria and the mosquito.
- D. Dengue and the mosquito.

INSECTS AND TICKS AS FACTORS IN TRANSMITTING DISEASE—

The mosquito.

The fly.

The ant.

The flea.

The bedbug.

The roach.

The tick, etc.

Texas fever—*Piroplasma bigeminum*.

Spotted fever of Bitter Root Valley. Mont.

Sleeping sickness—*Trypanosomiasis*.

TRYPANOSOMES—

Inoculate rat with *Trypanosoma Lewisi*.

Sleeping sickness,

Dourine.

Surra.

Nagana.

Mal de caderas.

Culture of trypanosomes on water of condensation in blood-ajar.

FILARIAL EMBRYOS (“*Filaria sanguinis hominis*”) in the blood.

Filaria Bancrofti Cobbold, 1877.

Filaria diurna Manson, 1891.

Filaria perstans Manson, 1891.

Filaria Demarquayii Manson, 1895.

Dracunculus mediensis (Linnæus, 1758). [Guinea worm.]

Relapsing fever. (*Spirocheta Obermeieri*.)

FERMENTS—

Organized.

Unorganized—enzymes.

A. *Amylolytic*:

Ptyalin.

Diastase.

Amylopsin.

Convert starch and glycogen into sugar.

B. *Proteolytic*:

Pepsin.

Trypsin.

Bacterial peptonizing enzymes.

Convert proteids into peptones.

C. *Steatolytic*—steapsin.

Split fats into fatty acids and glycerin.

D. *Invertin*—in intestinal juice and yeast cells.

Convert cane sugar, maltose, lactose into glucose.

E. *Coagulation*:

Rennet.

Fibrin ferment.

Convert soluble into insoluble ferments.

F. *Alcoholic ferments*:

Saccharomyces.

Converts carbohydrates into alcohol, carbon dioxid, etc.

Fermentation in—

Wine.

Beer.

Vinegar, acetic.

Milk:

Lactic.

Butyric.

Koumys.

Kefir.

IMMUNITY-

Natural.

Inherited.

Acquired:

Active.

Passive.

Toxin immunity:

Toxemia.

Antitoxins.

Bacterial immunity:

Bacteriemia.

Bacteriolysins.

Theories explaining immunity:

A. Alexins, or bactericidal substances in the blood.

Nuttall and Buchner.

B. Chemical theory—Ehrlich's side chains.

Antitoxin immunity:

Receptors.

Toxin—

Haptophore group.

Toxophore group.

Cytolytic immunity.

1. Complement, alexin or cytase; normally present, not specific for species, thermolabile.

2. Immune body, amboceptor, intermediary body, sensitizer, fixative, preparative, additive, desmon.

Produced by process of immunization, specific and thermostabile.

C. Biological theory—phagocytosis of Metchnikoff.

Ameboid motion.

Digestive vacuoles—formation of acid.

Phagocytes:

Free phagocytes—

Microphages—

Polymorphonuclear neutrophiles.

Eosinophiles.

Macrophages—

Large mononuclear lymphocytes.

Transitional forms.

Epithelioid cells.

Fixed phagocytes—

Connective tissue corpuscles.

Vascular endothelium.

Kupfer's cells, etc.

Small lymphocytes and mast cells lack ameboid motion and are not phagocytic.

Chemotaxis—

Positive.

Negative.

D. Enzyme theory—immunizing proteid of Emerich and Löw.

E. Retention theory.

F. Exhaustion theory.

TOXINS—

Toxins, toxones, toxoids, etc.:

a. Ptomaines.

b. Leucomaines.

c. Tox-albumens.

d. Enzymes.

Ricin (castor bean).

Abrin (jaquerity bean).

Snake venom.

CYTOLYSINS AND CYTOLYSIS—

- a.* Bacteriolysins.
- b.* Hemolysins.
- c.* Leucolysins.
- d.* Spermatolysins, etc.

TRANSPORTATION OF DISEASED TISSUES AND BACTERIOLOGIC SPECIMENS, ETC., BY MAIL AND EXPRESS.

The Regulations of the Post-Office Department, Order No. 176, March 2, 1900.
Mailing cases.

Methods of sending dry specimens.

Methods of sending moist specimens.

Methods of preparing and forwarding samples for bacteriologic study.

Methods of preparing and forwarding samples for histologic study.

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TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.
WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 9.

M. J. ROSENAU, Director.

SEPTEMBER, 1902.

PRESENCE OF TETANUS

IN

COMMERCIAL GELATIN

BY

JOHN F. ANDERSON.



WASHINGTON:
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PRESENCE OF TETANUS IN COMMERCIAL GELATIN.

[BY JOHN F. ANDERSON, ASSISTANT SURGEON, UNITED STATES PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.]

Gelatin injections are now used rather extensively, in Europe especially, as a means of treatment for aneurism and as a direct and prophylactic hemostatic. It is generally used in a 2 per cent solution in water, boiled immediately before use, and, as a hemostatic, injected subcutaneously. There seems a great variation in the amount of solution used, some using 200 c. c. and others as high as 1,000 c. c.

Gelatin is a substance obtained from the nitrogenous portions of bone, hides, connective tissue, etc. The collagen when boiled is changed to gelatin. Glue is impure gelatin to which has been added some preservative and some substance to increase its power of adhesion. Bone and hide gelatin are the two principal kinds of gelatin. Isinglass is gelatin made from the bladders of sturgeons. According to Whipple (5), the general process of manufacturing hide gelatin is as follows:

The hide scraps are first macerated and subjected to the action of a solution of lime or caustic soda in pits for two or three weeks. This dissolves most of the blood and saponifies the fats. The excess of lime or soda is then largely removed by washing and the solution steamed to dissolve the gelatin, but an excess of heat is avoided. Sulphurous acid is used to bleach the gelatin. When of sufficient strength, the gelatin is allowed to harden in molds or on slabs, and is ultimately dried in sheets on wire nets. Bone gelatin is made in a somewhat similar manner. The bones are crushed, boiled, treated with hydrochloric acid, and the gelatin is dissolved as before, washed, bleached, and dried in sheets. The process requires a number of weeks.

According to the National Dispensatory, gelatin is made by boiling in water for a long time bone cartilage, animal skin, tendons, and ligaments. These substances become soluble in the boiling water, and when the mass is allowed to cool, form a jelly. The animal tissues are placed upon a sieve or perforated diaphragm some distance from the bottom of the boiler. When the solution has become sufficiently saturated it is drawn off and allowed to cool, and the jelly is then cut into thin sheets and placed on wire nettings to dry. While so exposed to the air for drying it could easily become infected with tetanus spores, as the gelatin is often manufactured in conjunction with the other procedures that go on in a large meat-packing house.

Kuhn (1) reported a case of tetanus following the injection of gelatin in a hemophiliac.

Gerulanos, Georgi, and Lorenzo (2) report four cases of tetanus following the injection of gelatin for the purpose of arresting hemorrhage

after operations such as for subphrenic abscess and for disease of the urinary tract. In one of these last four cases the organism was obtained from the seat of injection.

Drs. Hochhalt and Herezel (3) report a case of tetanus on the seventh day after an injection of 2 per cent gelatin following splenectomy.

Dr. Laszlo Deatsch (3) studied bacteriologically the gelatin used in the last-named case, and found that after being boiled for five minutes and then grown anaerobically it contained an anaerobic, spore-bearing bacillus. He advises that gelatin, to be used as a hemostatic, be inoculated with bacillus subtilis before sterilization, and, as this organism is more resistant to heat than that of tetanus, if there is no growth of subtilis the gelatin can be used without fear of tetanus.

Drs. Levi and Brun (4), of Strasbourg, examined six samples of gelatin and in four found tetanus bacilli. They found that the spores of the tetanus bacilli which they isolated were able to resist streaming steam for a longer period than eight minutes.

Six different makes of gelatin were purchased in the open market and, with the stock laboratory gelatin, an investigation was begun with regard to the presence of tetanus in the same. Ten grams of each sample of gelatin were placed in 100 c. c. of glucose bouillon and heated at 50° C. until dissolved, then rendered slightly alkaline to litmus and heated to 80° C. for ten minutes to endeavor to kill all nonspore-bearing organisms; the flasks at the end of ten minutes were quickly placed in a Novy jar and boiled in vacuo to expel all of the air; the jars were then placed in the incubator and kept at 37° C. for one week. On the seventh day the flasks were removed from the jars. Three of the flasks remained sterile; of the other four, three showed an end-spore bearing rod, the spores of two of them being oval, and the third round with a very fine rod; this last stained by Gram.

The same day .5 c. c. of each culture was injected subcutaneously into white rats, but beyond making them very sensitive to sudden noises for one or two days, no results were noted. Two days later .5 c. c. of the culture containing the round, end spore rod was put into a white mouse. The mouse was found dead the next morning. Bouillon tubes were inoculated from the site of inoculation and with the heart's blood, but both remained sterile. Two drops of this culture, which we will call No. 1, were put into a tube of bouillon, heated to 80° C. for five minutes, and from this glucose agar plates were made and incubated for seven days at 37° C. in a Novy jar. At the end of a week two of the plates showed deep, oval colonies; stained smears showed these to be a fine rod with a round end-spore.

A flask and three tubes of *freshly* made bouillon were inoculated from the plates; the flask was heated to 80° C. for ten minutes; the tubes were not heated after inoculation. Flask and tubes were grown in a Novy jar as before. One drop of the bouillon growth from one of the tubes was injected into the right side of a mouse; mouse died next

morning with signs of tetanus, such as stiff tail and retraction to right side. Smears made from the site of inoculation showed the presence of a thin rod with a round end-spore. Decreasing doses were put into white mice on successive days until finally it was found that .000022 c. c. would kill a mouse with typical symptoms of tetanus in four days, with an incubation period of about thirty-six hours

SUMMARY AND CONCLUSIONS.

Seven samples of gelatin examined; one showed tetanus spores.

Two samples showed an oval end-spore rod, whose identity was not proved, but in stained specimens it would be hard to distinguish from tetanus, if indeed not tetanus with diminished virulence.

In tetanus investigations it is important to use *freshly* made bouillon, as the organism is apt not to germinate in bouillon over ten days old.

The thermal death point of the organism isolated was found to be between twenty and thirty seconds at 100° C.

It is important, therefore, that gelatin to be used for injections should be boiled at least ten minutes on account of the variability of the thermal death point in different species of tetanus. Whether this amount heating impairs in any way the hemostatic power of gelatin has not been settled, but in case it does it is believed that the danger from tetanus more than overbalances its therapeutic value.

It is suggested that when, as in hospitals, there is likelihood of gelatin injections being used for hemostatic purposes the gelatin solution be sterilized by the fractional method on three successive days and kept ready for use in sterile containers.

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TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 10.

M. J. ROSENAU, Director.

February, 1903.

R E P O R T

UPON THE

PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF HOOKWORM DISEASE
(*Uncinariasis or Anchylostomiasis*)

IN THE

UNITED STATES.

BY

CH. WARDELL STILES, Ph. D.

Chief of Division of Zoology.



WASHINGTON:
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1903.

ORGANIZATION OF HYGIENIC LABORATORY.

WALTER WYMAN, *Surgeon-General*,
U. S. Public Health and Marine-Hospital Service.

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SUMMARY

Convinced from theoretical deductions that hookworm disease (uncinariasis) must be more or less common in the South, a trip was made from Washington, D. C., to Ocala, Fla., stopping at penitentiaries, mines, farms, asylums, schools, and factories, and the fact was established that the chief anemia of the Southern rural sand districts is due to uncinariasis, while clay districts and cities are not favorable to the development of this disease.

In the Old World, hookworm disease was probably known to the Egyptians nearly three thousand five hundred years ago, but its cause was not understood until about the middle of the nineteenth century, when it was shown to be due to an intestinal parasite, *Agchylostoma duodenale*. Until 1893 no authentic cases of this disease were recognized as such in the United States, but between 1893 and 1902 about 35 cases were diagnosed. In 1902 it was shown that a distinct hookworm, *Uncinaria americana*, infests man in this country, and this indicated very strongly that the disease must be present although not generally recognized. It is now established that in addition to the few cases of Old World hookworm disease imported into the United States we have in the South an endemic uncinariasis due to a distinct cause, *Uncinaria americana*. This disease has been known for years in the South and can be traced in medical writings as far back as 1808, but its nature was not understood. Some cases have been confused with malaria, others have been attributed to dirt-eating.

The hookworms are about half an inch long. They live in the small intestine, where they suck blood, produce minute hemorrhages, and in all probability also produce a substance which acts as a poison. They lay eggs which can not develop to maturity in the intestine. These ova escape with the feces and hatch in about twenty-four hours; the young worm sheds its skin twice and then is ready to infect man. Infection takes place through the mouth, either by the hands soiled with larvæ or by infected food. Infection through the drinking water may possibly occur. Finally, the larvæ may enter the body through the skin and eventually reach the small intestine.

Patients may be divided into light cases, in which the symptoms are very obscure; medium cases, in which the anemia is more or less marked, and severe cases, represented by the dwarfed, edematous, anemic dirt-eater. Infection occurs chiefly in rural sand districts. Above the frost line the symptoms are more severe in summer than in winter, and whites appear to be more severely affected than negroes. Persons who come in contact with damp earth are more commonly infected than others, so that the disease is found chiefly among farmers, miners, and brickmakers. Severe cases are more common in women and children than in men over 25 years of age. Uncinariasis is a disease which occurs in groups of cases, and if one case is found in a family the chances are that other members of the same family are infected.

The testimony of patients severely infected is unreliable. Recalling that any one or more symptoms may be absent or subject to variation, it may be noted that the period of incubation (at least before the malady can be diagnosed by finding the eggs) is from four to ten weeks. Stages are not necessarily distinctly defined, but are described as (1) stage of purely local symptoms, corresponding to the light cases; (2) stage of simple anemia, corresponding to the medium cases; and (3) dropsical stage, corresponding more or less to the severe cases. The duration of the disease after isolation from the source of infection has been traced for six years and seven

months: how much longer infection will last is not established. If a patient is subject to cumulative infection, the disease may last five, ten, or even fifteen years, and in case of light infection perhaps longer.

External appearance.—In extreme cases there is a general lack of development; skin waxy white to yellow or tan; hair is found on the head, but is more or less absent from the body; breasts are undeveloped; nails white; external genitalia more or less rudimentary; face anxious, may be bloated; conjunctivæ pale; eyes more or less dry, pupil dilates readily; membranes pale according to the anemia; teeth often irregular; tongue frequently marked with purple or brown spots; cervical pulsations prominent; thorax emaciated; heart beats often visible; abdomen frequently with "pot belly;" extremities emaciated, frequently edematous, and with wounds or ulcers of long standing.

Urine 1010 to 1015; in advanced cases albumin without casts; acid or alkaline.

Feces reddish brown, contain eggs, and may contain blood.

Circulatory system.—Anemia pronounced, according to degree and duration of infection; blood watery, with decreased red blood corpuscles and with eosinophilia; "heart disease" very commonly complained of; hemic murmurs present; pulse 80 to 132 per minute.

Temperature.—Subnormal, normal, or to 101° or 102° F.

Respiratory system.—Breathing may be difficult, slow, or increased to as high as 30.

Muscular system.—Emaciation and great physical weakness.

Digestive system.—Appetite poor to ravenous; abnormal appetite often developed for pickles, lemons, salt, coffee, sand, clay, etc.; pain in epigastrium; constipation or diarrhea.

Nervous system.—Headache, dizziness, nervousness, mental lassitude, and stupidity.

Genital system.—Menstruation irregular or absent; if present, it occurs chiefly in winter; there is a marked tendency to abortion.

Diagnosis.—The safest plan is to make a microscopic examination of the feces to find the eggs; or, if feces are placed on white blotting paper, a blood-like stain will be noticed.

Treatment.—Thymol, or male fern (or ? calomel); iron, and good food.

Prognosis.—Good, if patient is not too far gone at time of treatment.

Lethality.—Not yet determined.

Prevention.—Treat all cases found and dispose of feces.

Economically, uncinariasis is very important. It keeps children from school, decreases capacity for both physical and mental labor, and is one of the most important factors in determining the present condition of the poorer whites of the sand and pine districts of the South.

The disease is carried from the farms to the cotton mills by the mill hands, but does not spread much in the mills; nevertheless, it causes a considerable amount of anemia among the operatives.

REPORT UPON THE PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF HOOKWORM DISEASE (UNCINARIASIS OR ANCHYLOSTOMIASIS) IN THE UNITED STATES.

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INTRODUCTION.

Thoroughly convinced from theoretical zoologic considerations, especially of a faunistic nature, that uncinariasis must be a more or less common disease in the Southern portion of the United States, I requested instructions from Surgeon-General Wyman to study the subject in a field investigation. The desired authorization was received and the results of the work are contained in this paper.

DEFINITION.

Uncinariasis is a specific zooparasitic disease found especially in tropical and subtropical sand areas, and caused by hookworms (genus *Uncinaria*) which inhabit the small intestine. Its chief symptoms are: Anemia, with the circulatory symptoms found in all extreme anemias, namely, dizziness, palpitation, hemic murmurs; great weakness, in some cases with considerable emaciation; colicky pains in the abdomen; perverted appetite, such as "dirt-eating;" constipation or diarrhea, stools sometimes brownish or bloody; nausea; edema. The only positive diagnosis is by finding the parasite or its eggs in the stools. It may affect any class of patients, but is more frequent in persons whose daily life brings them in contact with damp earth (children, farmers, miners, brickmakers, excavators, etc.).

TERMINOLOGY.

The disease now under discussion is known by a number of different names, but uncinariasis^a should be adopted as the more correct technical designation. Among the names frequently applied to it, the

^aLooss (1902) has recently attempted to suppress the term "uncinariasis" in favor of anchylostomiasis, his view being that the genus *Ancylostoma* is distinct from *Uncinaria*. His suggestion does not help matters much at present. Even if the zoological genera are recognized as distinct, uncinariasis would still exist in man, while among animals it would be still more common than anchylostomiasis. Further, the two genera would probably have to be united in a subfamily, which could then be called "Uncinariinae," and uncinariasis could then signify any infection of any

following may be mentioned in particular: Anchylostomiasis, ankylostomiasis, brickmakers' anemia, Egyptian chlorosis, miners' anemia, miners' cachexia, tunnel anemia, St. Gothard tunnel disease, tropical chlorosis, hookworm disease, and tunnel disease. (See also pp. 31, 32, 96.)

HISTORICAL REVIEW.

In order to understand the exact status of the subject of uncinariasis, it will be well to take a brief historical review of hookworms in general, hookworm disease in general, and hookworm disease in the United States.

BRIEF REVIEW OF HOOKWORMS.

It is quite probable that the ancient Egyptians, nearly thirty-five hundred years ago, were acquainted with the parasites which we now call hookworms. From a zoological standpoint, however, the first hookworm known to science was a parasite in the intestine of the common badger (*Meles taxus*) of Europe, described by Goeze, a German clergyman, in 1782. Goeze called the parasite "der Haarrundwurm" (the hair round worm), and gave to it the Latin name *Ascaris criniformis*. Although he placed this species in the same genus with the ordinary eelworm, *Ascaris lumbricoides*, he intimated that it represented a distinct genus. One of the anatomical characters which Goeze noticed was a membranous expansion on the tail of the male, and in this he saw two finger- or ray-like structures which he interpreted as "hooks" (see caudal rays in figs. 1, 15).

In 1789 Frœlich found a similar worm in the common fox (*Canis vulpes* or *Vulpes vulpes*) of Europe. He noticed the same membranous expansion and "two hooks with many points" on the end of the tail. On account of this character he adopted the vernacular name "Haakenwurm" (hookworm), and proposed the generic name *Uncinaria* for the new genus which he established.

It is now known that the membranous expansion is the caudal bursa, found in all members of the family Strongylidæ, while the so-called "hooks" represent the "rays" or "ribs" which support the bursa (see fig. 1). In the early part of the nineteenth century several other species of hookworms were described as parasitic in various animals, and they were united generically with the "colic worms" (strongyles) of horses.

animal with any member of this subfamily. In case the term "anchylostomiasis" is adopted, which of the many spellings should be recognized? Adopting uncinariasis relieves us of the necessity of discussing that point, and further gives to the name of the disease the same orthography in several different languages. The case at hand gives rise to the question whether it is not inadvisable to name diseases after the zoological names of the parasites, at least during the transitional stage of zoological nomenclature. As a matter of fact it is the function of the medical profession, not that of the zoological, to determine what names should be used to designate diseases, but at the present moment medical terminology is subjective.

In 1843 Dubini, of Milan, Italy, described a hookworm as parasitic in man. Besides the caudal "hooks" (i. e., the "rays") of the male, Dubini's parasite presented four hooks in the mouth. It presented further an anatomical character which is common to all hookworms, namely, the ventral surface of the anterior end grows more rapidly than the dorsal surface, so that the oral end is bent backward like a "hook," and the mouth thus occupies a dorsal position. Thus it is seen that the original character which led to the vernacular name "hookworm" was a misinterpretation; the second character of "hooks" (namely, in the mouth), which has been popularly but erroneously interpreted as responsible for the vernacular name "hookworm," is not present in all species; the hooklike curvature of the head is usually but not always distinct. I propose, however, to retain the word "hookworm" as a vernacular name.

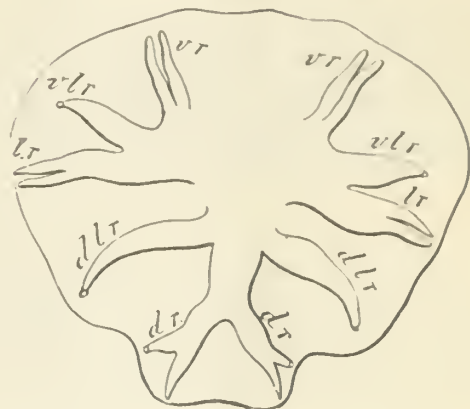


FIG. 1.—Caudal bursa of a male strongyle (*Esophagostoma dentatum*), to serve as diagram for the family: *v. r.*, ventral rays; *v. l. r.*, ventro-lateral rays; *l. r.*, lateral rays; *d. l. r.*, dorso-lateral rays; *d. r.*, dorsal rays. X 93. (After Schneider, 1866, p. 130.)

It is not apparent that Dubini knew that Frølich had proposed the genus *Uncinaria*, and it is probably on this account that he proposed a new genus—*Agchylostoma* by name—to contain the parasite (*Agchylostoma duodenale*) which he had found in man.

For years it was supposed that this was the only species of hookworm found in man, but in May, 1902, I showed that in America we have a distinct species, which I named *Uncinaria americana*.

ZOOLOGICAL POSITION OF THE PARASITES.

The parasites which cause uncinariasis are worms belonging to the nematode family Strongylidae.

Family STRONGYLIDÆ.

FAMILY DIAGNOSIS.—Nematoda: With body elongate, cylindrical, rarely filiform. Mouth is probably always provided with six papillæ, of which the four submedian are generally salient in form of nodules or conical points. In some cases the mouth is in the axis of the body; in others it is turned dorsally or ventrally, and occasionally provided with a chitinous armature. Esophagus more or less swollen in posterior portion, but without forming in adults a distinct esophageal bulb. Male provided with a caudal bursa, open or closed, entire or divided, and with one or two spicules. Female with one or two ovaries; vulva anterior or posterior of equatorial plane, in some cases near the anus. Eggs deposited during segmentation, in some cases containing embryo.

TYPE GENUS.—*Strongylus* O. F. Møller.

The sexes are separate and the digestive tract is complete. Characteristic for the family is the presence, on the tail of the male, of an umbrella-like structure known as the caudal or "copulatory bursa."

supported by a number of finger-like "rays," which may be compared to the ribs of an umbrella. In coitu, the male clasps the body of the female by means of this bursa.

This family is divided into subfamilies, according to the presence of certain anatomical characters. The parasites of uncinariasis are now classified in the subfamily Strongylinae,^a which, as its former name, Sclerostominae, indicated, is characterized by the presence of a hard chitinous "buccal capsule."

Subfamily STRONGYLINÆ.

SUBFAMILY DIAGNOSIS.—Strongylidæ: Meromyaria; mouth with more or less complete chitinous armature. Male with two equal spicules; caudal bursa with rays, the dorsomedian and dorsolateral being united in a common base. Female with two ovaries, except in *Ollulanus*.

TYPE GENUS.—*Strongylus* Mueller.

The subfamily Strongylinae is in turn divided into a number of genera, of which we may mention here the following:

Strongylus [*Sclerostoma*]; the sclerostomes, including the colic worms of horses and the kidney worms of hogs (but not the kidney worms of dogs and man);

Syngamus, including the gape worms of chickens; and

Uncinaria, the hookworms, including the parasite of uncinariasis.

It seems very probable that hookworms will have to be divided into several different genera, for which a new subfamily will perhaps be recognized, but it is not quite clear at present just what genera will be admitted. Undoubtedly *Uncinaria* Frœlich, 1789, must be adopted for one, and in this will probably be placed worms like *Uncinaria stenocephala*, possessing ventral lips but not ventral recurved teeth. It is quite possible that a second genus (*Monodontus* Molin, 1861, or *Bunostomum* Railliet, 1900) will be recognized for certain other forms, with buccal lips and with the prominent dorsomedian buccal tooth, as was proposed by Molin; probably the new American hookworm will be placed in this genus. Hookworms with the ventral recurved buccal teeth, as seen in *Uncinaria duodenalis* and *Uncinaria canina* will probably be separated into a distinct genus, for which Dubini's name *Agchylostoma* will be available. To satisfactorily determine the points at issue will require further anatomical study of a number of different species. For the purpose of this paper it will be sufficient to call attention to these probable changes.

^aFrom a study of the history of the nematode genera, it is very clear that there will have to be a general revision of the technical names of this group. The original *Strongylus*, for instance, was a sclerostome, hence the names *Sclerostoma* and *Sclerostominae* will have to fall into synonymy. It is probable that *Metastrongylus* will be the correct name for the lung strongyles. *Strongylus contortus* becomes *Hæmonchus contortus*. As soon as certain remaining points of this nature are decided, Hassall and I will issue a list of nematode genera, together with their type species.

Genus UNCINARIA^a Frœlich, 1789.

GENERIC DIAGNOSIS.—Strongylinae: With anterior extremity curved dorsally; mouth round to oval, opening oblique, limited by a transparent border and followed by a chitinous buccal capsule; the dorsal portion of the capsule is shorter than the ventral, and is supported by a conical structure, the point of which sometimes extends into the cavity; at the base of the buccal capsule are found two ventral teeth; toward the inner free border the ventral wall bears on each side of the median line chitinous structures, lips (*Uncinaria*) or teeth, often recurved in shape of hooks (*Agchylostoma*); the inner dorsal wall may also bear lips or teeth. Oviparous, eggs with thin, transparent shell.

TYPE SPECIES.—*Uncinaria vulpis*^b Frœlich, 1789.

^aSYNONYMY, WITH ORIGINAL PLACE OF PUBLICATION.

- 1789: *Uncinaria* FRÆLICH<Der Naturforscher, Halle, v. 24, pp. 130-139; type, *Uncinaria vulpis* Frœlich, 1789.
- 1799: *Unciaria* FISCHER<Arch. f. d. Physiol., Halle, v. 3, p. 99. [Apparently a misprint for *Uncinaria*.]
- 1843: *Agchylostoma* DUBINI<Annal. univers. di medic., Milano, v. 106, aprile, pp. 5-13; type, *Agchylostoma duodenale* Dubini, 1843.
- 1845: *Ancylostoma* CREPLIN<Archiv f. Naturg., Berlin, 11. J., v. 1, p. 325; for *Agchylostoma* Dubini, 1843.
- 1845: *Dochmius* DUJARDIN, Histoire naturelle d. helminthes, pp. 267, 275-279; type, *Uncinaria vulpis* Frœlich, 1789.
- 1845: *Docmius* DUJARDIN, ibidem, p. 114. [Misprint for *Dochmius*.]
- (1846): *Anchylostoma* DELLE CHIAJE<Rendicon. dell'Accad. delle Sci. Napoli, v. 5, p. 339. [Not verified.]
- 1850: *Anchylostoma* DUBINI, Entozoografia umana, pp. 102-112; for *Agchylostoma* Dubini, 1843.
- 1851: *Ancylostomum* DIESING, Systema helminthum, v. 2, p. 82; for *Agchylostoma* Dubini, 1843.
- 1851: *Anchylostomum* DIESING, Systema helminthum, v. 2, pp. 321-322; for *Agchylostoma* Dubini, 1843.
- 1861: *Monodontus* MOLIN (not *Monodonta* Lamarck, 1799), Il sottordine degli acrofialli <Mem. r. Ist. ven. di sc., lett. ed arti, Venezia, v. 9, pp. 435, 463-470; type, *M. semicircularis* Molin, 1861.
- 1861: *Dochmius* MOLIN<Ibidem, p. 471. [Misprint for *Dochmius*.]
- 1862: *Dachmius*<Veterinarian, Lond. (416), v. 35, 4. s. (92), v. 8, Aug., pp. 549-556. [Misprint for *Dochmius*.]
- 1879: *Anchylostoma* BOZZOLO<Osservatore, Torino, v. 15 (24), 17 giugno, pp. 369-370; for *Agchylostoma* Dubini, 1843.
- 1895: *Ankylostomum* STROSSICH<Boll. Soc. Adriatica di sc. nat. in Trieste, v. 16, pp. 21-25; for *Agchylostoma* Dubini, 1843.
- 18-?: "*Ankylostoma* Dubini" of various authors; for *Agchylostoma* Dubini, 1843.
- 1897: *Anchylostamum* MEHLAU<Buffalo M. J., v. 36 (8), Mar., p. 573. [Misprint for *Anchylostomum*.]
- 1902: *Dohmius* LOOSS<Centrabl. f. Bakteriöl., Parasitenk. [etc.], Jena, 1 Abt., v. 31 (9), 5. Apr., Originale, p. 424. [Misprint for *Dochmius*.]
- 1902: *Uncinaria* VON LINSTOW<Zool. Centralbl., Leipz., v. 9 (24-25), 16. Dec., p. 778. [Misprint for *Uncinaria*.]

^bThis species is probably identical with *Uncinaria melis* Frœlich, 1789; *Ascaris criniformis* Goeze, 1782, and with *Uncinaria stenocephala* (Railliet, 1884).

The anatomical character which distinguishes the genus *Uncinaria* is the dorsal curvature of the anterior extremity of the body, due to the shortness of the dorsal wall of the buccal capsule and resulting in bringing the mouth into a dorsal instead of a terminal or a ventral position.

In many medical writings this genus is named *Anchylostoma*, a word which is spelled in at least nine different ways, and the disease is spoken of as *anchylostomiasis*. This nomenclature and terminology are due to the fact that when the hookworm (*Uncinaria duodenalis*) of man was first described, in 1843, it was supposed to represent a new genus (*Agchylostoma*). As a matter of fact, however, it is generally acknowledged to be congeneric with a worm described in 1789 as *Uncinaria*. By the international "law of priority," therefore, the names *Agchylostoma*, *Anchylostoma*, etc., fall into synonymy until it can be shown that the two species are not congeneric. (See p. 14.)

In explanation to physicians it may be here stated that zoologists are obliged to deal with hundreds of thousands of technical names, and on this account they have been forced to adopt very rigid rules governing their use. Our most important rule is the "law of priority," which to us is as essential as is the "code of ethics" to the physician.

The genus *Uncinaria* contains blood-sucking worms of the worst type. They are usually not over an inch in length nor thicker than an ordinary hatpin. They are provided with a heavy armature of sharp teeth, by means of which they pierce the intestinal mucosa of their host. They have also an unusually strong muscular esophagus, which serves as a pump during the act of sucking blood. An important point, from the medical aspect of the parasites, is that they do not remain fastened to one spot in the bowels, but suck first at one spot and then at another. Thus the patient loses blood directly to the parasites, and also, by numerous minute hemorrhages, into the intestine. It is probably this latter factor which occasionally gives to the stools of patients that peculiar reddish-brown tinge, and also their occasional bloody appearance.

The injury to the intestinal wall does not stop with the bite. The wound forms an excellent point of attack for bacteria, and the intestinal wall becomes thickened, thus losing, to a greater or lesser degree, the ability properly to perform its function. Not only does the patient lose blood, but his power of assimilation is impaired, and the supply of blood-forming material is thus in part cut off. Some authors also claim that the parasites produce a poison which acts upon the system, a view which is very strongly supported by certain clinical facts.

It was stated above that hookworms are found in various animals. Now, the general rule may be laid down that where these worms are present trouble may be expected.

Uncinaria americana and *Agchylostoma duodenale* cause in man the disease variously known as uncinariasis, uncinariosis, anchylostomiasis, tunnel disease, miners' anemia, brickmakers' anemia, mountain anemia, etc.

Agchylostoma caninum [*Uncinaria canina*^a] causes a similar disease in dogs, resulting, in some parts of the country, in a death rate of from 25 to 40 per cent of the pups born. Uncinariasis in dogs is exceedingly common in Washington, D. C. "Typhoid" in cats is attributed to this parasite.

Instructors in medical colleges who wish to demonstrate hookworms and their eggs to the students will find *A. caninum* of dogs an excellent substitute for *Agchylostoma duodenale* of man in case the latter species can not be obtained.

Uncinaria stenocephala occurs in dogs, foxes, and allied animals, and is causing considerable trouble in the blue fox (*Vulpes lagopus*) industry.

Uncinaria trigonocephala^b is found in sheep and produces a serious anemia. This parasite has been met in Victoria and Calhoun counties, Tex., where, in conjunction with the twisted wireworm (*Hæmonchus contortus*^c), it has caused the death of from 25 to 50 per cent of certain flocks.

Uncinaria radiata^d is found in cattle, producing trouble just below the stomach. The writer has collected this parasite in Dewitt, Gonzales, Victoria, and Calhoun counties, Texas, and has seen specimens from Florida collected by Dr. C. F. Dawson.

Uncinaria Lucasi was found several years ago in the seal pups of Alaska by Mr. Lucas, after whom the worm has been named. It is responsible for about 17 per cent of the deaths of the pups.

Still other species of hookworms are reported for other animals.

None of the species from animals mentioned above is known to affect man, nor has either *Uncinaria americana* or *Agchylostoma duodenale* of man been satisfactorily demonstrated to occur normally in other hosts than man.

^aThis is *Uncinaria canina* (Ercolani 1859) Railliet, 1900, a parasite of canines and felines, which is usually known as *Uncinaria trigonocephala* (Rudolphi, 1809) Railliet, 1885 [not *Uncinaria trigonocephala* (Rudolphi, 1809) Railliet, 1900]. If *Uncinaria* and *Agchylostoma* are recognized as distinct, *U. canina* should be placed in the same genus as *U. duodenalis*. I have not yet tested the correctness of the specific name *canina* for this form, but it is here accepted on authority of Railliet.

^bThis is *Uncinaria trigonocephala* (Rudolphi, 1809) Railliet, 1900 [not "*U. trigonocephala* Rudolphi, 1809)" Railliet, 1885]. Both Railliet and I have recently examined Rudolphi's original material, and it is unquestionably identical with *U. cernua* (Creplin, 1829) of sheep. This species is closely related to *U. americana*.

^c*Strongylus contortus* Rudolphi.

^d*Bunostomum phlebotomum* Railliet.

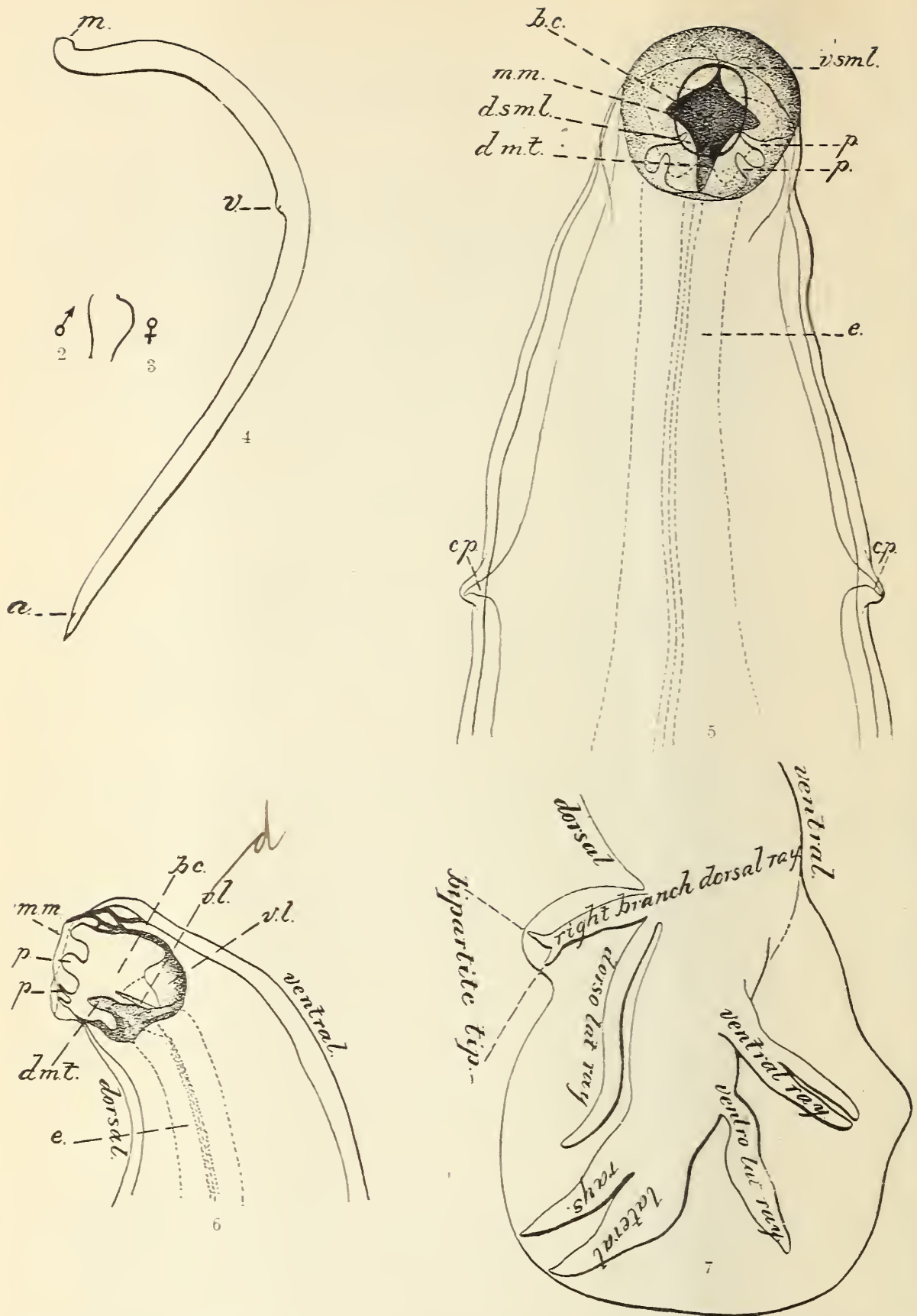


FIG. 2.—New World male hookworm (*Uncinaria americana*). Natural size. (After Stiles, 1902b, p. 190, fig. 120.)

FIG. 3.—New World female hookworm (*Uncinaria americana*). Natural size. (After Stiles, 1902b, p. 190, fig. 121.)

FIG. 4.—The same, enlarged to show the position of the anus (*a.*) and the vulva (*v.*). After Stiles, 1902b, p. 190, fig. 122.)

FIG. 5.—Dorsal view of anterior end of New World hookworm (*Uncinaria americana*): *b. c.*, buccal cavity; *c. p.*, cervical papillae; *d. m. t.*, dorsal median tooth, projecting prominently into the buccal cavity; *d. s. m. l.*, small dorsal semilunar lip; *e.*, esophagus; *m. m.*, margin of mouth, the prominent oval opening seen upon high focus; *p. p.*, papillae; *v. s. m. l.*, large ventral semilunar lips homologous with the ventral hooks of *A. duodenale*. Greatly enlarged. (After Stiles, 1902b, p. 190, fig. 123.)

FIG. 6.—Lateral view of anterior end of New World hookworm (*Uncinaria americana*): *b. c.*, buccal cavity; *d. m. t.*, dorsal median tooth, projecting prominently into buccal cavity; *e.*, esophagus; *m. m.*, margin of mouth; *p. p.*, papillae; *v. l.*, ventral lancets at the base of the buccal cavity, the left lancet is seen *en face*, the right lancet is seen from the side. Greatly enlarged. (After Stiles, 1902b, p. 190, fig. 124.)

FIG. 7.—Lateral view of caudal bursa of the New World male hookworm (*Uncinaria americana*), showing the arrangement of the rays. Note the short dorsal lobe. Greatly enlarged. (After Stiles, 1902b, p. 190, fig. 125.)

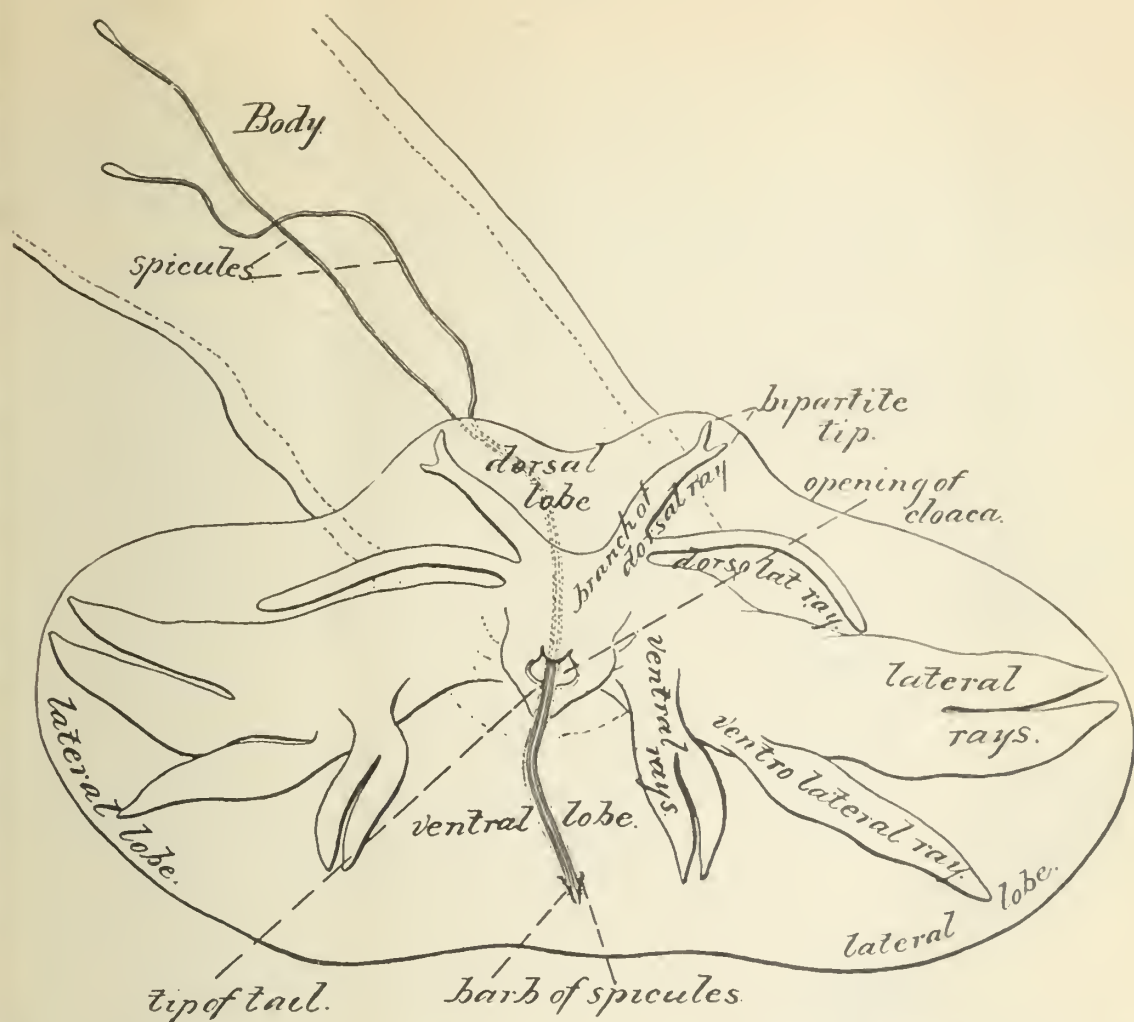


FIG. 8.—The caudal end of the New World male hookworm (*Uncinaria americana*). The bursa is spread out to show the arrangement of the rays. Note the short dorsal lobe which is subdivided, forming two lobes; note also the indistinct ventral lobe connecting the two lateral lobes. The dorsal lobe is thrown back over the body. Greatly enlarged. (After Stiles, 1902b, p. 191, fig. 126.)

[**UNCINARIA** Frœlich, 1789, *sensu stricto*].

DIAGNOSIS.—*Uncinaria* s. l. with buccal lips.

TYPE SPECIES.—*Uncinaria vulpis* Frœlich.

The New World hookworm—**UNCINARIA AMERICANA**^a Stiles, 1902—of man.

(Figures 2 to 9.)

SPECIFIC DIAGNOSIS.—*Uncinaria*: Body cylindrical, somewhat attenuated anteriorly. Buccal capsule with a dorsal pair of prominent semilunar plates or lips, similar to *U. stenocephala*, and a ventral pair of slightly developed lips of the same nature; dorsal conical median tooth projects prominently into the buccal cavity, similar to *Monodontus*; ♂ Male, 7 to 9 mm. long; caudal bursa with short dorso-median lobe, which often appears as if it were divided into two lobes, and with prominent lateral lobes united ventrally by an indistinct ventral lobe; for rays, see figures 7-8; common base of dorsal and dorso-lateral rays very short; dorsal ray divided to its base, its two branches being prominently divergent and their tips being bipartite; spicules long and slender. Female, 9 to 11 mm. long; vulva in anterior half of body, but near equator. Eggs, ellipsoid, 64 to 76 μ long by 36 to 40 μ broad, in some cases partially segmented in utero, in other (rare) cases containing a fully developed embryo when oviposited.

HABITAT.—Small intestine of man (*Homo sapiens*) in America (determined to date, for Virginia, North and South Carolina, Georgia, Florida, Alabama, Texas, Porto Rico, Cuba, and Brazil).

TYPE SPECIMENS.—No. 3310, B. A. I., U. S. Dept. Agric.

^aSYNONYMY WITH ORIGINAL PLACE OF PUBLICATION.

1902: *Uncinaria americana* STILES — Am. Med., Phila., v. 3 (19), May 10, pp. 777-778.

1902: *Uncinaria americana* (Stiles) von LINSTOW — Zool. Centralbl., Leipz., v. 9 (24-25), 16. Dec., p. 778. [Misprint.]

✓ 2 dorsal and a ventral pair of deep lancets

LIFE HISTORY OF *UNCINARIA AMERICANA*.

The life history of the American hookworm has not yet been determined in detail, but there is no reason for assuming that it will differ radically from that of *Agchylostoma duodenale* (see p. 24.)

In my first description of the worm I stated that the egg in the uterus may occasionally contain an embryo. Since making this observation on the females sent to me by Dr. Allen J. Smith I have examined hundreds of fresh eggs, but have not found any containing developed embryos. The question may therefore legitimately arise whether the females originally examined were not exposed to the air for some time before they were preserved; thus making the development of the eggs possible. If the embryo does develop in the uterus, as indicated by some of Dr. Allen J. Smith's material, such an occurrence is undoubtedly rare.

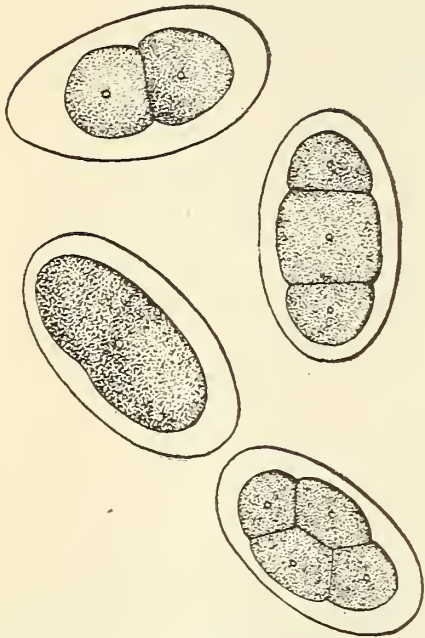


FIG. 9.—Four eggs of the New World hookworm, *Uncinaria americana*, in the 1, 2, and 4 cell stages. The egg showing 3 cells is a lateral view of a 4-cell stage. These eggs are found in the feces of patients and give a positive diagnosis of infection. Greatly enlarged. (After Stiles, 1902b, p. 192, fig. 127.)

DEVELOPMENT OUTSIDE THE BODY.

Segmentation.—In feces exposed to a September, October, or early November temperature of the Carolinas, Georgia, and Florida, the embryo develops in the egg (fig. 9) in about one day's time. In some instances the embryo develops in less than 24 hours. It is a common occurrence to find feces 24 hours old containing free embryos.

The conditions under which the trip was made were not favorable to exact observation in regard to temperature, moisture, etc. It was, however, possible to find worms in their first ecdysis about 2 to 3 days after hatching, and worms in the second ecdysis about 7 to 9 days after hatching. These observations were made under most unfavorable circumstances, when careful measurements, drawings, etc., were excluded, hence they should be repeated.

DEVELOPMENT INSIDE THE BODY.

Experimental infections during the trip were, of course, impossible.

[AGCHYLOSTOMA^a Dubini, 1843.]

DIAGNOSIS.—*Uncinaria*: Provided with ventral recurved teeth.

TYPE SPECIES.—*Agchylostoma duodenale* Dubini, 1843.

The Old World hookworm—AGCHYLOSTOMA DUODENALE^b Dubini, 1843, or UNCINARIA DUODENALIS (Dubini) Railliet, 1885—of man.

(Figures 10 to 41.)

SPECIFIC DIAGNOSIS.—*Agchylostoma*: Body cylindrical, somewhat attenuated anteriorly. Buccal cavity with two pairs of ventral teeth curved like hooks, and one pair of dorsal teeth directed forward; dorsal rib not projecting into the cavity. Male, 8 to 11 mm. long; caudal bursa with dorso-median lobe, and prominent lateral lobes united by a ventral lobe; for rays, see fig. 14; dorsal ray divides at a point two-thirds its length from its base, each branch being tridigitate; spicules long and slender. Female, 10 to 18 mm. long; vulva at or near posterior third of body. Eggs, ellipsoid, 52 to 60 μ by 32 μ , laid in segmentation. Development direct without intermediate host.

^aThere are numerous ways of spelling this word (see p. 15), more than one with more or less philological authority. Under these circumstances I adopt the original orthography, despite the fact that it is not philologically correct. By this action I do not intend to necessarily reject the ruling covered by the International Code, but from practical experience I find it impracticable to carry out said rule in reference to the emendation of names until the question of homonyms is decided.

^bSYNONYMY, WITH ORIGINAL PLACE OF PUBLICATION.

1843: *Agchylostoma duodenale* DUBINI <Ann. univ. di med., Milano, v. 106, aprile, pp. 5-13, pl. 1, figs. 1-5; pl. 2, figs. 1-3.

1845: *Ancylostoma duodenale* (Dubini) CREPLIN <Arch. f. Naturg., Berl., 11. J., v. 1, p. 325.

(1846): *Ancylostoma duodenale* (Dubini) DELLE CHIAIE <Rendicon. dell' Accad. delle sci., Napoli, v. 5, p. 339. [Not verified.]

1850: *Ancylostoma duodenale* (Dubini) DUBINI, Entozoografia umana, pp. 103-112.

1851: *Ancylostomum duodenale* (Dubini) DIESING, Systema helminthum, v. 2, p. 322.

?(1851): *Strongylus quadridentatus* SIEBOLD <Naturforsch. Versamml. z. Gotha. [Not verified.]

1861: *Dochmius anchylostomum* MOLIN, Il sottordine degli acrofalli <Mem. r. Ist. ven. di sc., lett. ed arti, Venezia, v. 9, pp. 485-487.

1864: *Sclerostoma duodenale* (Dubini) COBBOLD, Entozoa, pp. 361-362, fig. 77.

1866: *Strongylus duodenalis* (Dubini) SCHNEIDER, Monographie der Nematoden, Berl., pp. 139-140, 1 fig., pl. 9, fig. 3.

1866: "*Ancylostomum duodenale* Dubini" of WHITE <Boston M. & S. J., v. 75 (21), Dec. 20, p. 427. [Misprint for *duodenale*.]

1876: *Dochmius duodenalis* (Dubini) LERCKART, Die menschlichen Parasiten, v. 2 (3), pp. 410-460, figs. 235-239, 241-247, 249.

1879: *Ancylostoma duodenale* (Dubini) BOZZOLO <Osservatore, Torino, v. 15 (24), 17 giugno, pp. 369-370.

1881: *Dochmius duodenalis* <Rev. méd. de la Suisse Rom., Genève, v. 1 (3), 15 mars, p. 190.

1885: *Uncinaria duodenalis* (Dubini) RAILLIET, Éléments de zool. méd. et agric., Par., pp. 357-359, figs. 245-248.

1897: *Ancylostomum duodenale* (Dubini) MEHLER <Buffalo M. J., v. 36 (8), Mar., pp. 573-579. [Misprint for *Ancylostomum duodenale*.]

Ancylostoma duodenale and *Ancylostomum duodenale* of various authors.

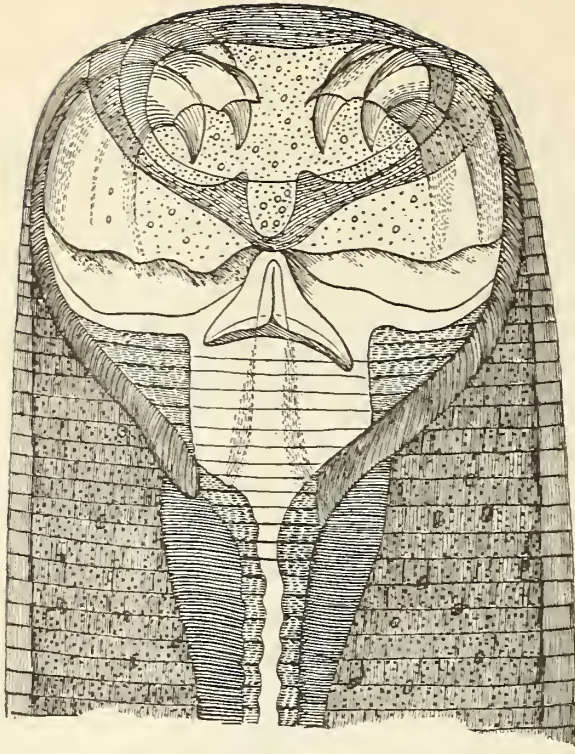


FIG. 10.—Dorsal view of anterior end of the Old World hookworm (*Agchylostoma duodenale*) of man. Greatly enlarged. (After Perroncito, 1882, p. 339, fig. 140.)



FIGS. 11-12.—Old World male and female hookworms (*Agchylostoma duodenale*) of man. Natural size. (After Stiles, 1902b, p. 187, figs. 115, 116.)

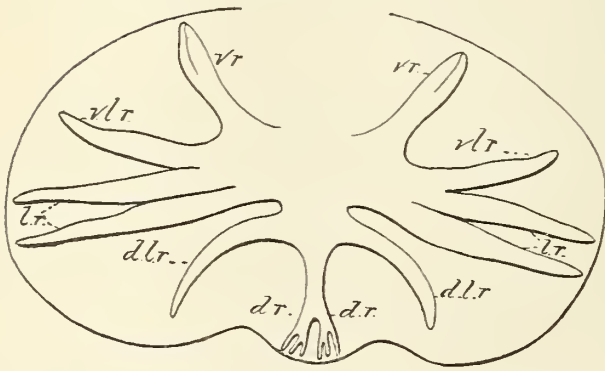


FIG. 14.—Semidiagrammatic figure of the caudal bursa of an Old World male hookworm (*Agchylostoma duodenale*) of man. (After Railliet, 1886, p. 357, fig. 247.)

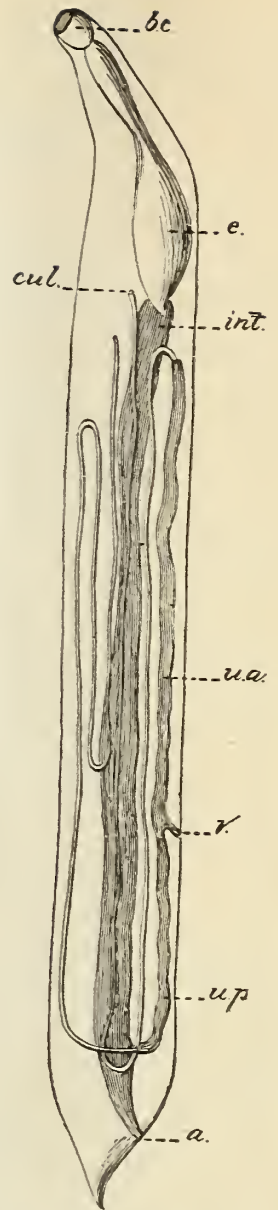


FIG. 13.—Old World female hookworm (*Agchylostoma duodenale*) of man, greatly enlarged diagram to show the anatomy: a., anus; b. c., buccal capsule; cul., cul de sac of ovary; e., esophagus; int., intestine; u. a., anterior uterus; u. p., posterior uterus; v., vulva and vagina. (After Schulthess [copied from Blanchard, 1888a, p. 761, fig. 374].)

HABITAT.—In small intestine of man (*Homo sapiens*); also alleged to occur in certain apes. Africa, Europe, Asia, Philippines, introduced into America.

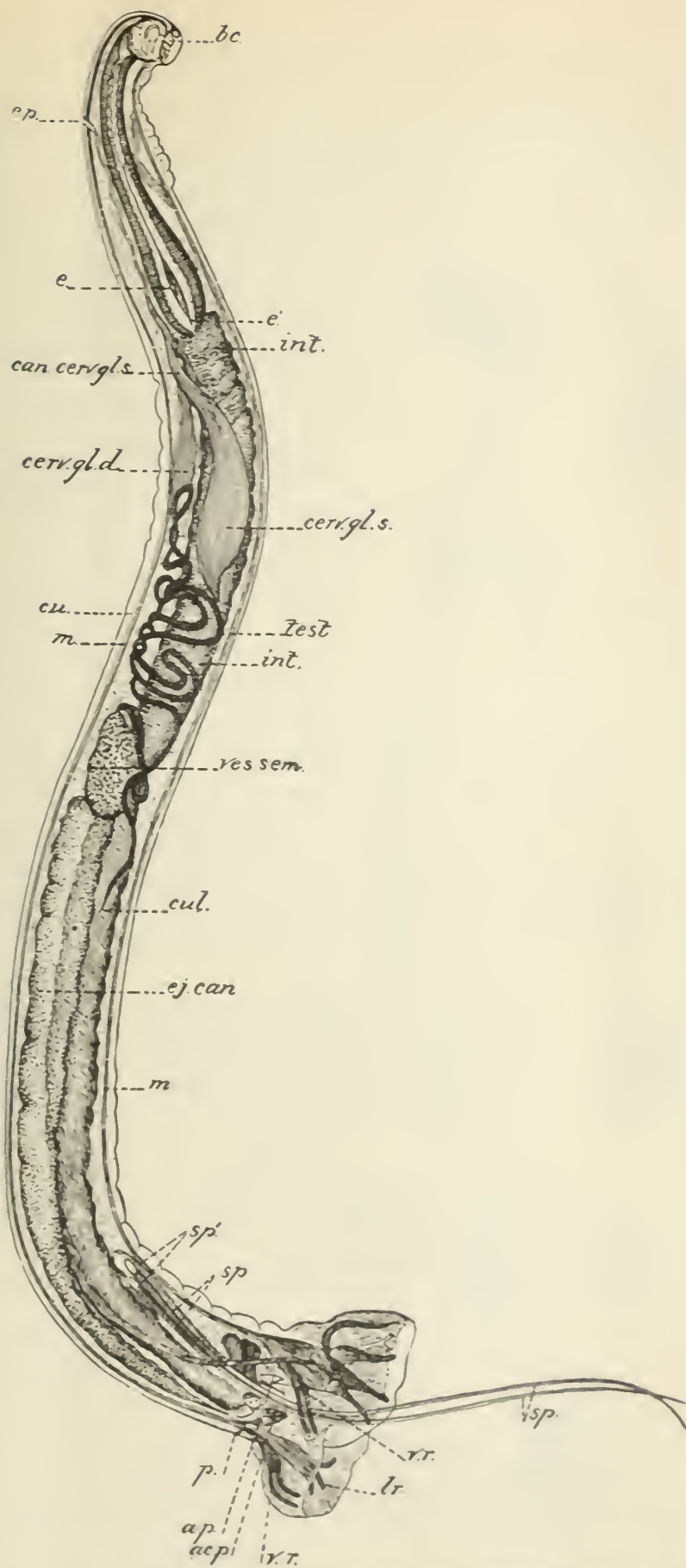


FIG. 15.—Male hookworm (*Ancylostoma duodenale*) of man: *ac. p.*, accessory piece to spicules; *a. p.*, "anal papilla;" *b. c.*, buccal capsule; *can. cerv. gl. s.*, canal of left cervical gland; *cerv. gl. d.*, right cervical gland; *cerv. gl. s.*, left cervical gland; *cu.*, cuticle; *cul.*, cul de sac of testicular tube; *e.*, esophagus; *e'*, posterior end of esophagus; *e. p.*, ventromedian excretory pore; *ej. can.*, ejaculatory canal; *int.*, intestine; *l. r.*, lateral ray of bursa; *m.*, muscular layer; *p.*, lateral pre-caudal papilla; *sp.*, spicules; *sp'*, anterior end of spicules; *test.*, testicular tube; *ves. sem.*, vesicula seminalis; *v. r.*, ventral rays of bursa. Greatly enlarged. (After Schulthess [copied from Blanchard, 1888a, p. 755, fig. 370].)

LIFE HISTORY OF AGCHYLOSTOMA DUODENALE OR UNCINARIA DUODENALIS.

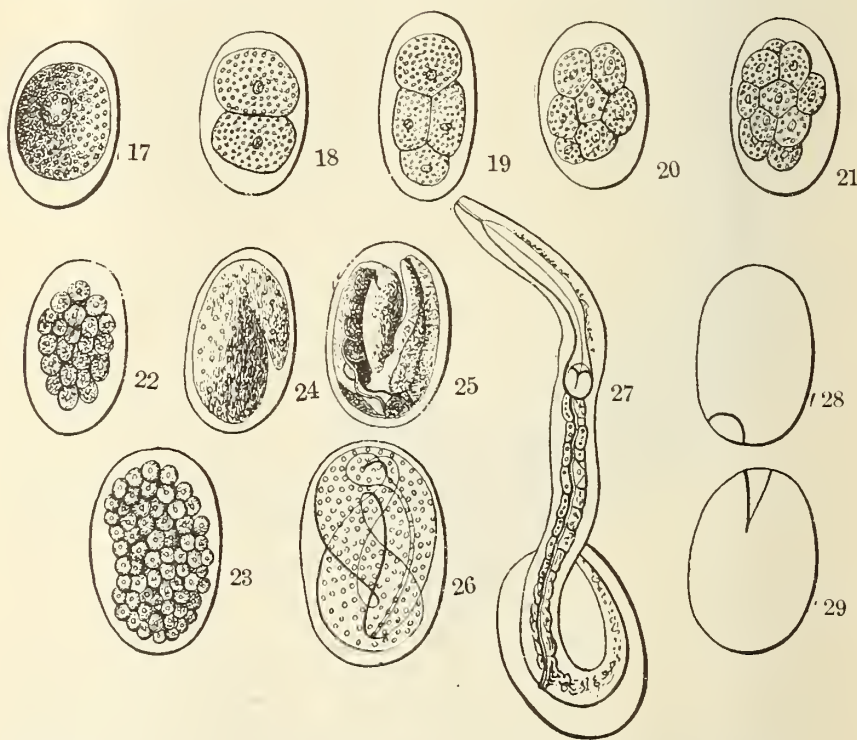
The eggs (fig. 16) are laid in the intestinal tract of the patient by the female worms and are discharged in the feces, either unsegmented or during the early stages of segmentation. They will not develop into adult worms in the intestine, but must first pass out of the body. Thus, for every adult hookworm present in the bowels a separate germ must enter the body.

The egg has a thin shell, which is an indication of a simple life cycle. A short time after escaping in the feces—the time varying according to temperature, moisture, and position in the feces—each egg develops (figs. 17–27) a minute embryo, which is known as a rhabditiform embryo (fig. 27). This name is given to it because of its resemblance to

worms of the genus *Rhabditis*. Characteristic for this stage is the rhabditiform esophagus, which is entirely different from the esophagus



FIG. 16.—Eggs of Old World hookworms (*Agchylostoma duodenale*) as found in the stools. Greatly enlarged. (After Stiles, 1902b, p. 193, fig. 128.)



FIGS 17-29.—Embryology of the Old World hookworm (*Agchylostoma duodenale*) of man; 17-23, segmentation of the egg, 24-26, the embryo; 27, a rhabditiform embryo escaping from its eggshell; 28-29, empty eggshells. Greatly enlarged. (After Perroncito, 1882, p. 342, fig. 142.)

of the adult hookworm. This embryonal esophagus is more or less bottle shaped, and consists of three parts—an anterior elongated

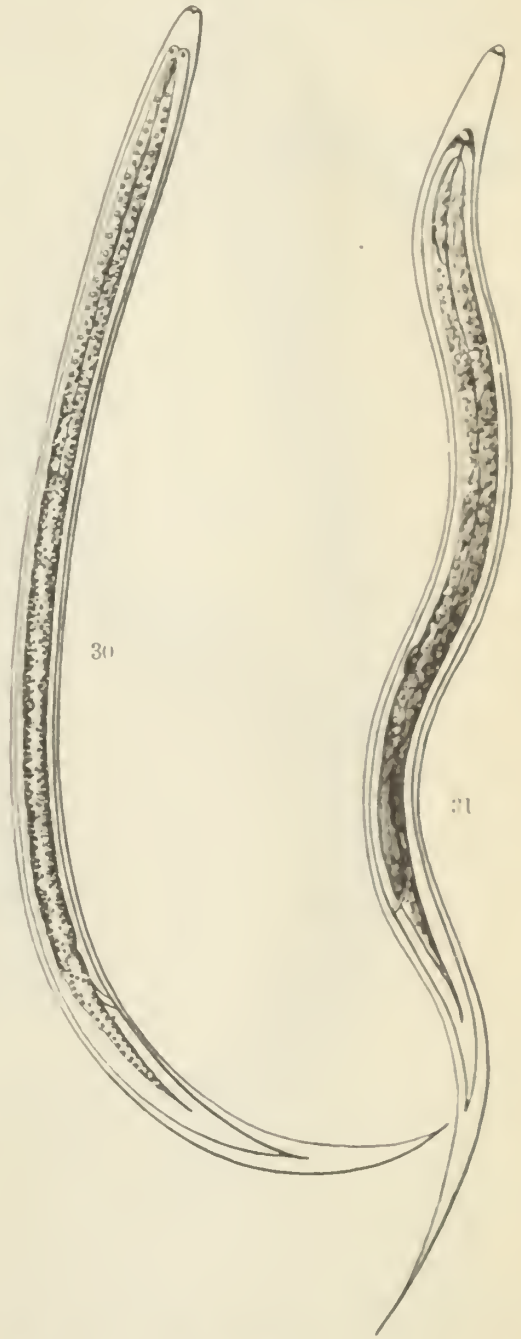
swollen portion, followed by a thin middle portion, the latter being followed by a more or less globular esophageal bulb which possesses a triradiate chitinous armature. This kind of esophagus is common to the early stage of all members of the family Strongylidae and also to numerous other free-living or parasitic nematodes. It is evidently a worm with an esophagus of this sort which was recently found in the earth taken from the New York tunnel excavations, and upon which was based the report that uncinariasis was present.

The embryo of the hookworm lives in water or moist ground. In its evolution the worm casts its skin four times, thus passing through five stages, and changes its structure so as to assume more and more the characters of the adult. During these changes the sexes become differentiated. Some of these changes occur in water or moist ground, and the rest after infection takes place.

DEVELOPMENT OUTSIDE THE BODY.

Segmentation.—The eggs develop best in the unaltered fecal matter, especially when this is well formed; not so well when it is more fluid in character. The addition of water retards the development, and if considerable water is added the eggs perish. Air is necessary to development, and the eggs nearer the surface of the feces segment more rapidly than those in the center. At a temperature of about 27° C. the embryo may form and escape from the shell in twenty-four hours. Lower temperatures retard development, so that at 21° or 22° C. the embryo may not escape for from thirty-six to forty hours; 1° C. kills the eggs in twenty-four to forty-eight hours, so that freezing weather may be looked upon as disinfecting areas exposed to the cold.

Embryo.—Upon escaping from the shell, the embryo (fig. 27) meas-



FIGS. 30-31.—Two larvae of the Old World hookworm at the end of the second stage ("encysted larva"), representing the young worms retracted from their skin. (After Perroncito, 1882, p. 350, figs. 148 a-b.)

ures 0.3 mm. in length; the anterior end is blunt, the tail long and pointed; 6 points are visible around the mouth, and these develop later into the papillæ; the buccal cavity is $10\ \mu$ long, $1.4\ \mu$ in diameter, and possesses a highly refractive chitinous membrane; the anus is $50\ \mu$ from the tip of the tail; excretory pore $50\ \mu$ from anterior end; $160\ \mu$ from anterior end is seen the primordium of the genital system.

In this stage the embryo takes food and grows. About the second

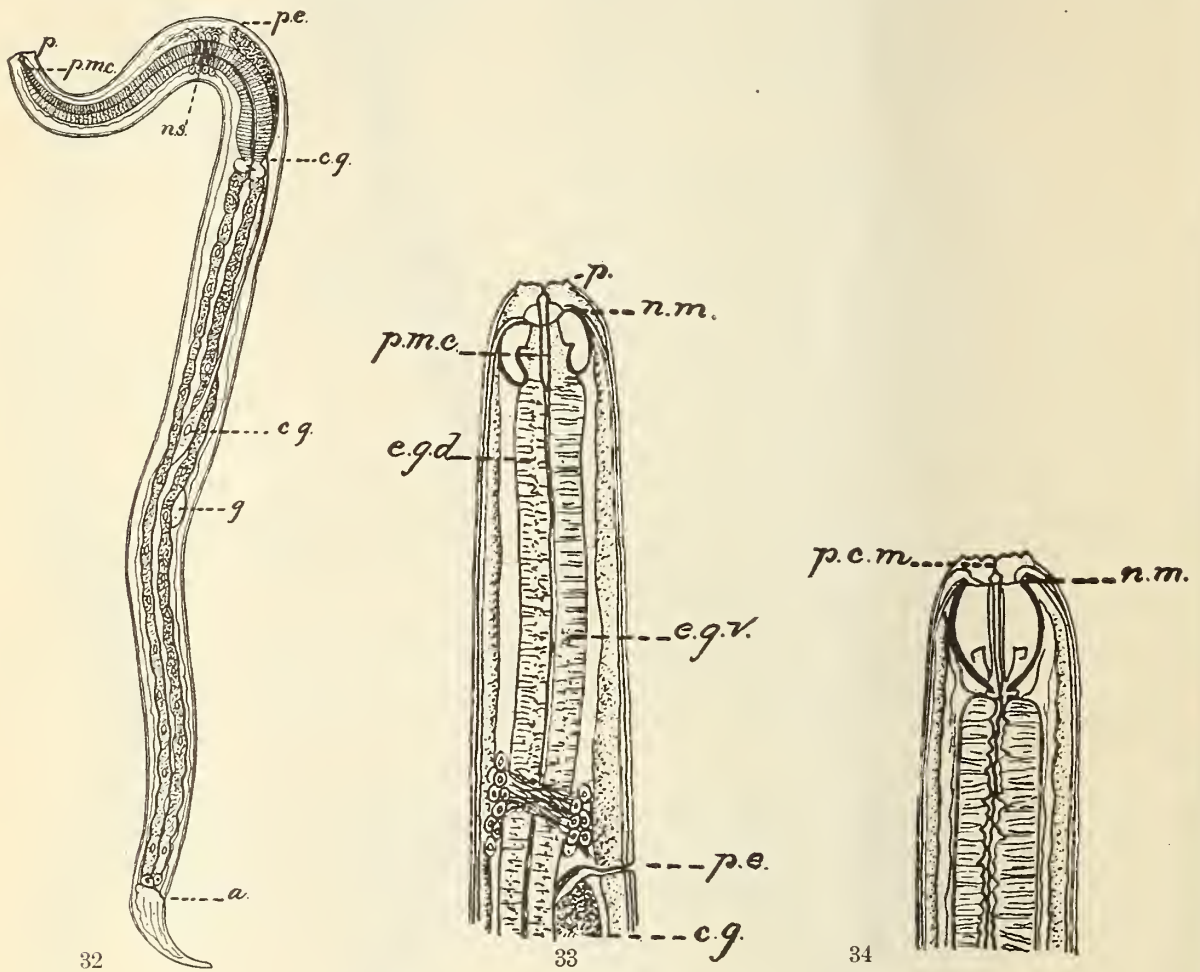


FIG. 32.—A young hookworm (*Agchylostoma duodenale*) of man, without buccal capsule, four days after infection: a, anus; c. g., cervical gland; g, primordium of genital organs; n. s., nervous system; p., papillæ on head; p. e., excretory pore; p. m. c., primary mouth cavity. X about 190 times. (After Looss, 1897, p. 919, fig. 1.)

FIG. 33.—Anterior end of a young hookworm (*Agchylostoma duodenale*) during formation of provisional buccal capsule: c. g., cervical gland; e. g. d., dorsal esophageal gland; e. g. v., ventral esophageal gland; n. m., new mouth; p., papillæ on head; p. e., excretory pore; p. m. c., primary mouth cavity. X 578. (After Looss, 1897, p. 920, fig. 2.)

FIG. 34.—Head of larval hookworm (*Agchylostoma duodenale*) before entering fourth stage, five or six days after infection; n. m., new mouth; p. c. m., primary mouth cavity, which extends through the provisional buccal capsule and continues as lumen of the esophagus. X 578. (After Looss, 1897, p. 921, fig. 3.)

or third day the embryo casts its first skin, but does not change its organization. After about four or five days (at 27°C.) it measures $480\ \mu$ long by $30\ \mu$ in diameter.

Second stage.—After the fifth day the young worms begin to show signs of a second ecdysis, at the same time undergoing certain other changes. Three minute lips, each with two very delicate papillæ, appear under the skin at the anterior end; the brightly refringent cutic-

ular lining of the buccal cavity and the chitinous teeth of the esophageal bulb disappear; the esophagus elongates, becomes thinner, and its three divisions become less distinct; the tail becomes slightly shorter and more blunt; the anus lies $90\ \mu$ from the tip of the tail. The organ-

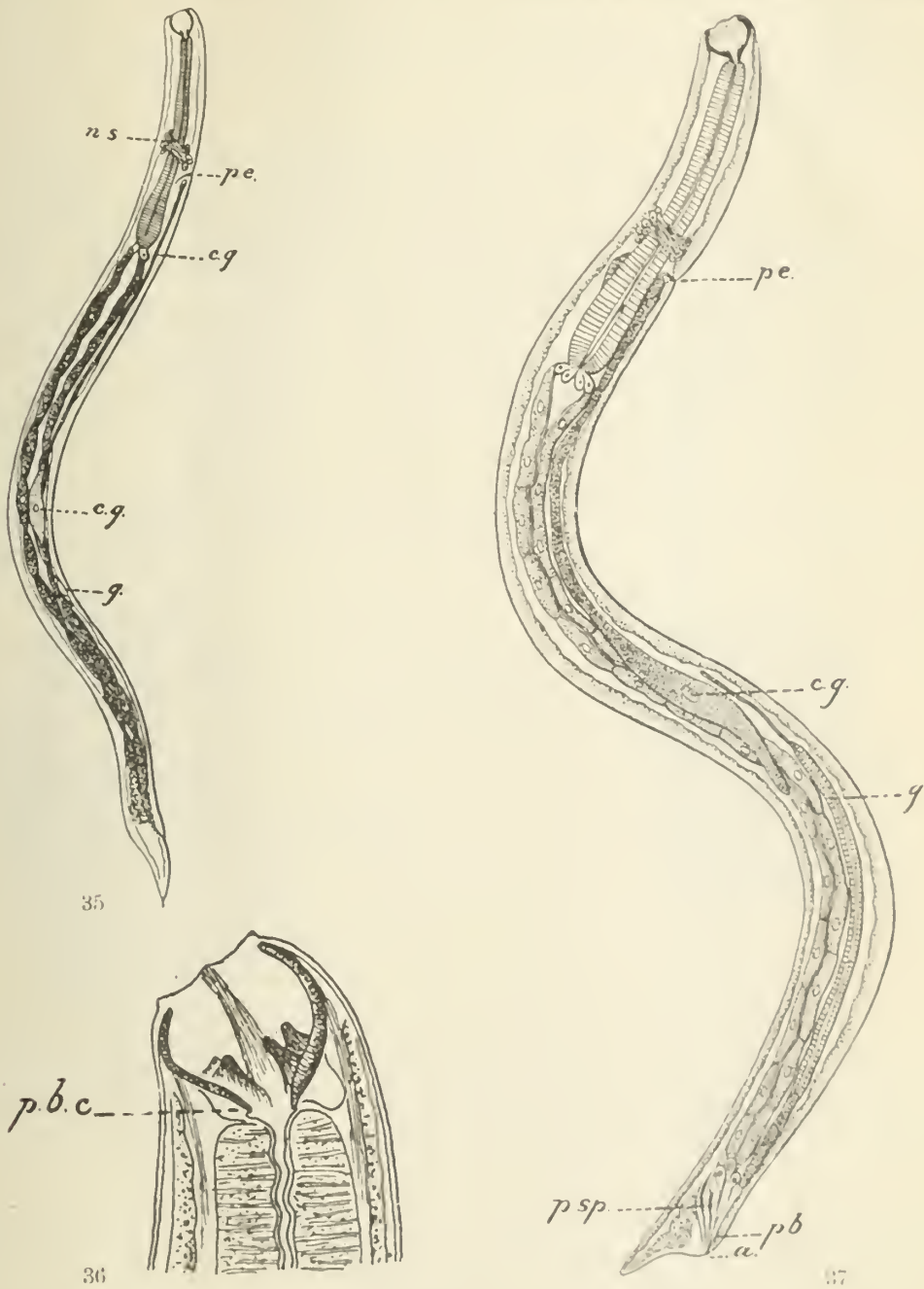


FIG. 35.—Young hookworm (*Agchylostoma duodenale*) in fourth stage, with provisional buccal capsule; *c. g.*, cervical gland; *g.*, primordium of genital organs; *n. s.*, nervous system; *p. e.*, excretory pore. X 105. (After Looss, 1897, p. 921, fig. 4.)

FIG. 36.—Provisional buccal capsule (fourth stage) of a larval hookworm (*Agchylostoma duodenale*), about nine days after infection; *p. b. c.*, primordium of definite buccal capsule. X about 150. (After Looss, 1897, p. 921, fig. 5.)

FIG. 37.—Young male hookworm (*Agchylostoma duodenale*), nine days after infection; *a.*, anus; *c. g.*, cervical gland; *g.*, genital tract; *p. b.*, primordium of bursa; *p. e.*, excretory canal; *p. sp.*, primordium of spicules. X about 105. (After Looss, 1897, p. 922, fig. 6.)

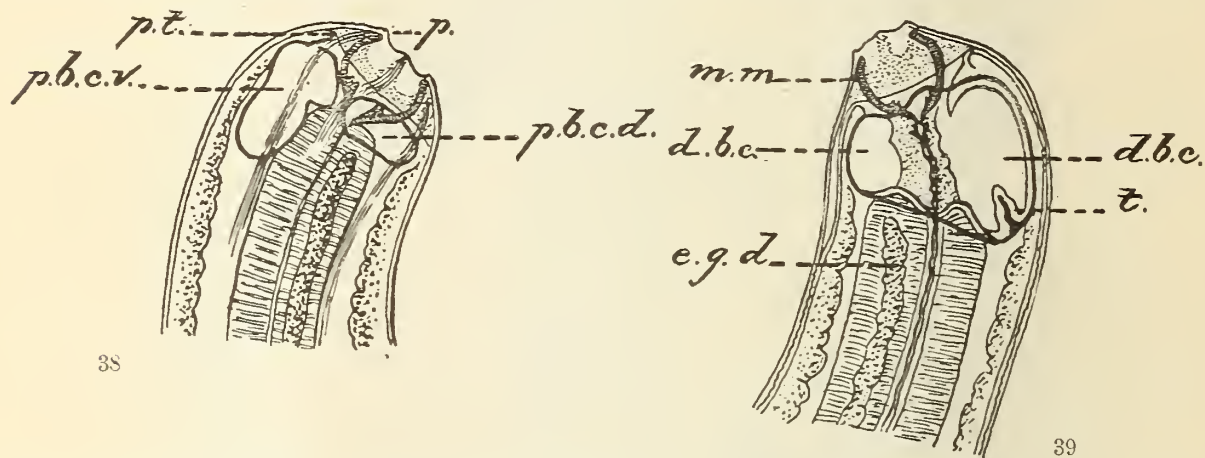
ism becomes more motile, and contracts from its outer skin, thus forming the stage (figs. 30–31) which has been described as an “encystation,” but which in reality is simply a second ecdysis. This is the infecting stage of the hookworm, and ends the development so far as the free life is concerned. No more food is taken. In some cases, however,

the worm escapes from the surrounding cast skin. While water is more or less injurious to the egg and the first stage, the infecting "encysted" stage exists well in this medium, and Looss (1897) succeeded in keeping these worms alive for thirty days in water. Upon drying up the larvæ die, so that the view that the worms exist in dust and are carried around in the air, thus leading to infection, is not well founded (see p. 30).

DEVELOPMENT INSIDE THE BODY.

Upon being swallowed these young worms undergo further ecdyses, changing their internal organization at the same time. We may recognize, with Looss, a third stage, without buccal capsule (fig. 32); a fourth stage, with a provisional buccal capsule (fig. 35); finally, a fifth stage, with the definite buccal capsule, corresponding to the adult form.

Third stage (without buccal capsule, fig. 32).—During their free life the larvæ may attain 0.65 to 0.7 mm. in length by 25 to 27 μ in diam-



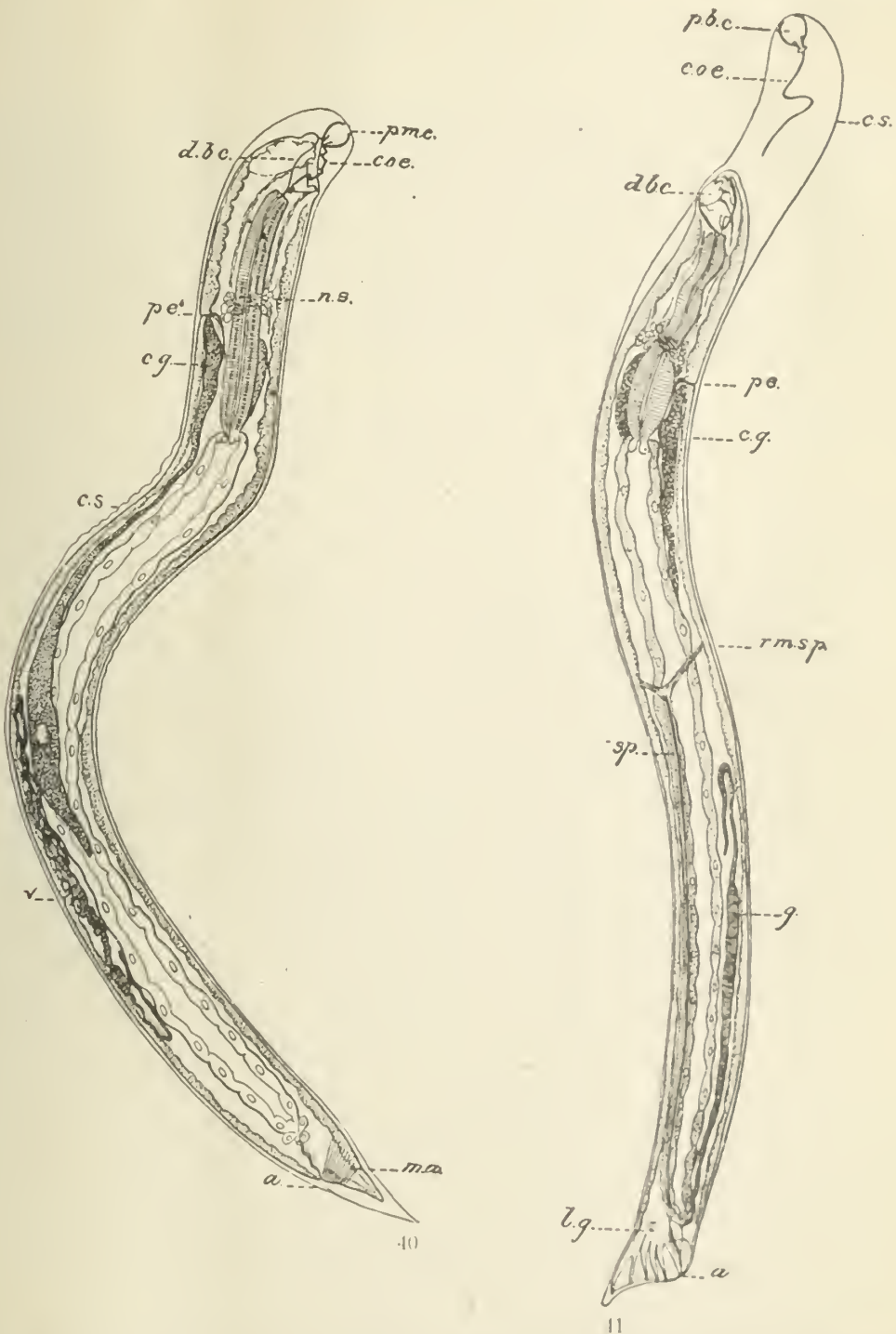
FIGS. 38-39.—Development of definite buccal capsule (38, on twelfth day after infection; 39, one or two days later): *d. b. c.*, definite buccal capsule; *e. g. d.*, dorsal esophageal gland; *m. m.*, margin of definite mouth; *p.*, papillæ on head; *p. b. c. d.*, *p. b. c. v.*, dorsal and ventral primordia of the definite buccal capsule; *p. t.*, primordium of ventral tooth; *t.*, ventral tooth; the new cuticle can be distinguished under the old. X 190. (After Looss, 1897, p. 923, figs. 7-8.)

eter (at the end of the esophagus). The esophagus is 160 μ long, and its three divisions may still be distinguished. The intestine is composed of about 15 rows of two cells each.

Fifteen hours after infection of dogs the worms have passed below the stomach. They now begin to feed, but their growth is comparatively slow. After about five days they begin to show signs of a third ecdysis, which continues until about the seventh day. During this period important changes take place, especially at the anterior end, and result in the formation of the provisional buccal capsule.

Fourth stage (with provisional buccal capsule, fig. 35).—This is the fourth larval stage, namely, the stage after the third ecdysis. The worms have not increased notably in length, but certain organs are advanced in development, and the esophagus no longer shows its former three divisions. The worms measure about 0.66 mm. long by 25 μ in diameter, the latter being nearly uniform for a greater part of the

length (fig. 35). The provisional buccal capsule attains $40\ \mu$ in diameter, and the mouth is bent slightly dorsad. Two pairs of teeth are visible at the base of the capsule—one pair situated dorsally, the other ventrally. During this stage the animal increases in length and thick-



FIGS. 40-41.—Male and female hookworms (*Agchylostoma duodenale*) during the fourth ecdysis of skin 14 to 15 days after infection; *a.*, anus; *c. g.*, cervical gland; *c. o. e.*, enticle of old esophagus; *c. s.*, cast skin; *d. b. c.*, definite buccal capsule; *g.*, genital organs; *l. g.*, large ganglion, supplying the rays of the bursa; *m. a.*, anal muscle; *n. c. gl.*, nucleus of cervical gland; *p. e.*, excretory pore; *p. b. c.*, provisional buccal capsule; *r. m. sp.*, retractor muscles of spicules; *sp.*, spicules; *v.*, vulva. X 42. (After Looss, 1897, p. 924, figs. 9-10.)

ness, the inner organs become better developed, the sexes become differentiated, and the definite buccal capsule forms at the anterior end. With these changes the parasite prepares for its last—namely a fourth—ecdysis, which occurs about fourteen to fifteen days after infection.

Fifth stage (with definite buccal capsule).—The worm is now about 1.9 (male) to 2 mm. (female) long, 12 to 14 μ in diameter—very much smaller than the adult forms. It is estimated that the parasites require about four to five or six weeks from the time of infection to become mature.

SOURCE OF INFECTION OF UNCINARIASIS.

The worms may be swallowed in contaminated food or in drinking water during or after the second ecdysis. Persons handling dirt are especially apt to get the microscopic worms on their hands, and it is an easy matter to transfer them to the mouth, either directly by biting the finger nails or sucking the fingers, or indirectly with food. In prevention, therefore, careful personal habits and pure drinking water are indicated.

Some writers state that the young stages are scattered in the air by the wind and in a dry state, the inference being that they may then be breathed in or may contaminate food. While not caring to go to the extreme of stating that such a method of dissemination or infection is impossible, my own observations on this class of parasites do not lead me to attach any importance to aërial infection. As a rule, drying-out results in a high mortality among nematode larvæ, embryos, and eggs, while moisture, on the other hand, is necessary for their existence. Now, uncinariasis is not so common as to fill the air with dried larvæ, and the chances of inhaling the latter appear to be almost infinitesimally small. Since the worms do not increase in number in the intestinal tract, we should therefore expect (in case aërial infection were common) to find rather few cases of severe infection, but a more or less uniform light infection of nearly all persons or animals inhabiting an infected area, since all are breathing the same air. Further, as adults breathe more air than children we should expect the former to present the cases of heaviest infection. We should also expect to find the disease more general in dry years than in wet seasons. Such, however, does not appear to be the case. Cases of infection vary greatly in intensity, and the losses from nematode diseases in sheep are much less in dry years and in dry localities than in wet seasons. As a matter of fact, few factors can be conceived of which would probably result in killing more germs of the disease than would dry winds.

In preventing uncinariasis and nematode diseases in general among live stock, systematic draining and burning of pastures are strongly advocated.

Looss (1898, 1901) recently suggested that the larvæ may enter the human body by way of the skin and then pass through the body to the intestine. Startling as this view is, Looss (see Sandwith, 1902) has recently demonstrated the correctness of it. (See p. 59.)

BRIEF REVIEW OF UNCINARIASIS.

Uncinariasis is by no means a new disease. According to Sandwith (1894), a medical papyrus, written about three thousand four hundred and fifty years ago, embraced in an encyclopedic form the knowledge at that time of Egyptian teachers. This oldest of all books among medical works (Eber's papyrus) came into the hands of Professor Ebers at Thebes, in 1873, and has recently been translated into German. Dr. Joachim (1890) and Scheuthauer (1881) agree that anemia, due to hookworms (*Ancylostoma duodenale*), was well known to physicians of those days under the name of "AAA" and "UIA." The papyrus describes accurately among the symptoms, "heart weakness, palpitation, stabbing cardiac pains, constipation, edema of the legs, a weight in the body pressing heavily, and other digestive troubles." It further prescribes a remedy for a patient who has in his body worms, which are produced by the "AAA" disease, and possibly it is the hookworms which are referred to.

Within modern times this special form of anemia was described in Brazil by Piso in 1648; Labat (1742 or 1748) observed it in Guadeloupe, Chevalier (1752) in St. Domingo, Dazille and Bason (1776) in the Antilles, and Edwards (1790 or 1793) in Jamaica. In Europe, the disease was first noted among the miners of Anzin in 1802.

Not until 1843 was the parasite (*Ancylostoma duodenale*) described, when Dubini of Milan published an account of it. Later it was reported from Egypt, Germany, France, India, Ceylon, Japan, Australia, and elsewhere, and to it was attributed a certain widespread anemia of brickmakers, tunnelers (St. Gothard tunnel anemia), and miners.

Zinn and Jacoby (1898), who have compiled 464 bibliographic references to the disease, give two charts showing its distribution at the time their paper was published.

In studying the maps, it will be well to recall that at the time they were printed nothing was known regarding the relations of uncinariasis to the soil (see p. 47), hence, the areas which are given as infected are probably much greater than the actual extent of the infested territory; further, the maps would indicate that they have registered the places in which hookworm disease has been diagnosed, and not necessarily the areas in which hookworm infection occurs.

In connection with their references to the United States, they simply mention Georgia, Alabama, and Louisiana, saying that there is little to report upon this subject for these localities.

BRIEF REVIEW OF UNCINARIASIS IN THE UNITED STATES.

In order to understand the American publications on this disease, it must be stated that much of the so-called "dirt-eating," "pica," "cachexia africana," antebellum "negro consumption," "mal d'estomac," "malnutrition," and "malarial anemia," described for the Southern Atlantic States, is in reality due to uncinariasis.

The earliest reference which seems quite positively to refer to this disease in this country, so far as I have yet found, is an article by Dr. Pitt (1808, pp. 340-341), who says that along the Roanoke River, North Carolina, malacia or dirt-eating "prevails mostly among the poor white people and negroes, and originates, in my opinion, from a deficiency of nourishment." Chabert's (1821) description of the conditions of the slaves of Louisiana, which he attributed to dirt-eating, Jordan's (1832, of Person County, N. C.) account of "cachexia africana, or negro consumption," Cotting's (1836) account of the dirt-eating in Richmond County, Ga., Little's (1845) description of the dirt-eaters of Florida, Le Conte's (1845) account of the dirt-eating in the pine barrens of Georgia, Duncan's (1850) record of dirt-eaters in St. Mary's Parish, La., all apply so well to the uncinariasis I studied in some of the same States that I have no hesitation in assuming that many, if not all, of the cases were due to infection with *Uncinaria*. Lethermann (Florida), Lyell (Georgia and Alabama), and Heusinger and Geddings (South Carolina) are said to have published on similar conditions in the South, but not being able to trace their articles, I am unfortunately unable to give them full credit for whatever views they may have advanced.

Blickhahn (1893a) seems to have been the first physician to recognize as such and to publish a case of uncinariasis for this country. The patient was a German brickmaker who had been in the United States seventeen months, and Blickhahn believes the infection took place in Germany. It is true that Herff (1894) records a case of supposed uncinariasis observed in Texas in 1864, and Allen J. Smith (published by Schaefer, 1901) found *Uncinaria* eggs in feces of man in Texas in 1893, but these publications are antedated^a by Blickhahn's article; hence Blickhahn has priority of discovery. It is, however, interesting to note that Blickhahn's case, being in a German, was probably caused by the Old World parasite, *Agchylostoma duodenale*, while the cases of Herff and Allen J. Smith were in all probability the first endemic cases recognized. Herff did not appear altogether certain regarding his diagnosis, but his short account of the worm indicates that the interpretation is correct.

^a In all zoologic matters neither priority of observation nor priority of presentation before a scientific society avails to give priority of discovery. By international agreement, and by custom extending back a century and a half, zoologists recognize only actual publication as governing a question of this sort.

Mœhlau (1896) reported five cases for Buffalo, N. Y., which were supposed to be due to the Old World parasite (*Agchylostoma duodenale*). Gray (1901) recorded two cases for Richmond, Va., contracted elsewhere in the same State, which he states positively (personal conversation) were due to *Agchylostoma duodenale*, and Tebault (1899) recorded a case of uncinariasis in a boy of German descent in New Orleans.

To one of my former pupils, Dr. Bailey K. Ashford (1900), of the U. S. Army, is unquestionably due the credit of having first seriously directed the attention of American physicians and zoologists to this disease.^a Ashford in fact found this malady very common in Porto Rico, and although he erroneously considered the parasite to be identical with the Old World species, his clinical observations placed the American medical profession on its guard for cases which might occur in returning American troops.

In 1901 there was a sudden increase in American observations. Dyer (March 15, 1901) reported a case for St. Louis. Schaefer (May, 1901) was reported in the proceedings of the Texas Medical Association (Texas Medical News, May, 1901) as presenting a paper on a new form of intestinal parasite in Texas. The case in question was uncinariasis, probably due to *Uncinaria americana*, but was not published as uncinariasis until October 26, 1901.

Claytor's (June, 1901) case is, so far as I am aware, the first published American case which can be recognized as unquestionably due to *Uncinaria americana*, although at least some (and probably most, if not all) of Ashford's cases were caused by this species. Claytor's case was originally published as an infection with the Old World species, *Agchylostoma duodenale* (an error for which the responsibility rests upon me, not upon Dr. Claytor; the large-sized egg found should have placed me more on my guard).

Allyn and Behrend (July 13, 1901) recorded an imported case in an Italian boy in Philadelphia, due apparently to *Agchylostoma duodenale*, and at the same time mentioned three unpublished cases diagnosed by Dr. L. Napoleon Boston, two in 1900 and one in 1901. All three cases came from Porto Rico, so that they were probably due to *Uncinaria americana*.

^aIt is true, as has been stated, that for some years prior to Ashford's publication I had repeatedly insisted upon the probability of the frequent occurrence of this disease in the United States, having discussed the subject in my lectures on medical zoology in the post-graduate medical school of the U. S. Army, and in the medical classes of Johns Hopkins University and of Georgetown University (the latter, the alma mater of Dr. Ashford), as well as in various medical meetings; but so far as I am aware, my views were not printed until July, 1901, so that Ashford's printed statements antedate mine by more than a year. Furthermore, his paper was a practical demonstration, while my views were theoretical deductions.

In a paper (July, 1901) written for the Texas Medical News I expressed very positive views to the effect that uncinariasis in man must be more or less widespread in the United States, and I discussed the disease in general. The position taken was based upon general zoologic principles, and, so far as I am aware, was the first printed definite claim regarding the frequency or probable frequency of the malady in the United States.

Schaefer (October 26, 1901) next published a case for Galveston, Tex., probably infected in Mexico, and made the very important statement that Allen J. Smith had found one case in Galveston in 1893, and since then that he had encountered two [afterwards six additional] cases among some 80-odd medical students of the University of Texas.

The importance of this discovery by Allen J. Smith should not be underestimated. To the clinician it did not mean very much, since no record existed that the students exhibited any very severe symptoms. To the zoologist, however, it meant a practical demonstration that uncinariasis was more or less common in the South. Here were 3 [9] students in a city (Galveston); the chances that the infection took place in Galveston did not seem very great; as the students came from different places (according to personal information), the infection must be more or less widespread; and since light cases occurred among medical students, heavier infections must naturally occur among persons who come more regularly in contact with the dirt. Allen J. Smith's observations, the importance of which has not yet been duly recognized by medical journals or by his colleagues, led to some correspondence between himself and me, and he very kindly forwarded specimens from one of his cases. In some respects these parasites resembled *Uncinaria stenocephala* of the dog, and both Allen J. Smith and I were fully agreed that they were not identical with *Agchylostoma duodenale*. I obtained specimens from Claytor's case and also some material which Ashford had sent from Porto Rico to the U. S. Army Medical Museum. All three lots of worms agreed with each other, and differed from *Uncinaria stenocephala*, which I had obtained from Europe, as well as from *U. trigonocephala*^a from sheep, *U. radiata* from cattle, *U. Lucasi* from the Alaskan seal, and from every other species of *Uncinaria* of which I could obtain either specimens or description. Accordingly, I described (May 10, 1902) these worms as a new species, naming it *Uncinaria americana*.

Having now an endemic species, with specimens in my possession for Washington, D. C. (patient came from Virginia), Porto Rico (Ashford's material), Cuba (specimens sent by Guiteras), and Galveston (Allen J. Smith's material), I did not hesitate to state positively in

^a *U. cernua* (Creplin, 1829). See Stiles, 1902b, p. 189.

a paper published in the Eighteenth Annual Report of the Bureau of Animal Industry, and issued on September 25, 1902, that we must have in the United States an endemic uncinariasis which had been generally overlooked.

Prior to the appearance of the paper in question, Claude Smith, of Atlanta, Ga. (June, 1902), had presented a case of uncinariasis before the American Medical Association (see below, p. 103), the parasite afterwards proving to belong to the species *Uncinaria americana*.

H. F. Harris (July 19, 1902), also published a case of uncinariasis (see below, p. 103) for Georgia. In reply to a letter from me, he stated (August 9) that he had found other cases also, and that he was "absolutely sure this disease is very common in this [Porter Springs] locality."

Upon my transfer from the United States Bureau of Animal Industry to the United States Public Health and Marine-Hospital Service, I decided upon an early study of uncinariasis in man, to determine its frequency and geographic distribution in the Southern States. A preliminary report (Stiles, 1902c, October 24) was published, giving my results for Virginia, North Carolina, and part of South Carolina. This report reads as follows:

[Reports to the Surgeon-General Public Health and Marine-Hospital Service.]

HOOKWORM DISEASE IN THE SOUTH—FREQUENCY OF INFECTION BY THE PARASITE
(*UNCINARIA AMERICANA*) IN RURAL DISTRICTS.

(Preliminary report by Dr. Ch. Wardell Stiles, chief of Division of Zoology, United States Public Health and Marine-Hospital Service, detailed for this investigation.)

KERSHAW, S. C., October 22, 1902.

SIR: Through the director of the Hygienic Laboratory, I have the honor to submit a brief preliminary report in regard to the frequency and geographic distribution of hookworm disease (uncinariasis) in the Southern States.

Meeting the disease in the Virgilina copper-mine district of southern Virginia and northern North Carolina, I have thus far traced it through the coal-mine district of Cumnock, N. C., the brickyards of Camden, S. C., and the granite-sand district of Lancaster and Kershaw counties, S. C.

The present indications are that it is more prevalent in sandy regions than in clay or stone districts. On the farms and plantations of the sand region of the two counties just mentioned, it appears to be the most common disease of man, and from an economic point of view it appears to be of great importance. The extreme cases seem to occur more commonly among children and women than among adult males over 25, but the present facts at my disposal do not indicate that the malady is quite so fatal as the European form of the disease caused by *Uncinaria duodenalis*. All of the cases thus far examined are due to *Uncinaria americana*, demonstrating clearly that this is an endemic infection and totally independent of the cases which have been introduced from Europe, Asia, and northern Africa.

In general, it may be said that the "pale skin," the "heart disease," the "diarrhea," the "bloat," and the suppression of menses which I have thus far examined all represented various stages of heavy infections with *Uncinaria americana*, and it is impossible to escape the conclusion that so far as the farms and plantations

are concerned a radical change in the general therapeutics practiced in the localities in question is urgently indicated.

As for the economic side of the problem, it should be recalled that the disease in question is resulting in loss of wages, loss in productiveness of the farms, loss in the school attendance of the children, extra expenses for drugs and for physicians' services, etc.

The heavy and frequent infections found are amply explained by the almost total absence of privies and closets on the farms visited. Defecation occurs at almost any place within a radius of 50 meters from the house or hut, and as a result the premises become heavily infested with the embryos.

The disease as thus far traced is primarily a "poor man's" malady, and in frequency it far exceeds even the most extreme limit which theoretical deductions seemed to justify before commencing the field work. There is, in fact, not the slightest room for doubt that uncinariasis is one of the most important and most common diseases of this part of the South, especially on farms and plantations in sandy districts, and indications are not entirely lacking that much of the trouble popularly attributed to "dirt-eating," "resin-chewing," and even some of the proverbial laziness of the poorer classes of the white population are in reality various manifestations of uncinariasis.

The infection among the miners, so far as discovered, is less severe and less common than the infection on the farms and plantations of the sandy districts.

Respectfully,

CH. WARDELL STILES, Ph. D.,
Chief of Division of Zoology.

On November 15, Dr. H. F. Harris, of Atlanta, Ga., published an important notice regarding uncinariasis in the South. After referring to his first case (see above, p. 35), he says:

"The discovery of a distinct American species of the hookworm is very important, as it leads to the inference that the aborigines of this country were infested with this parasite, and that the worm is probably present in all parts of the United States where the conditions are suitable for its development.

"My observations during the last six months bear out this assumption in a most striking manner. A few weeks after my first case of the disease was seen, a second one was encountered that originated in middle Georgia, but though I was constantly on the search for it no other case was found among the numerous patients that come to the clinics of the Atlanta College of Physicians. In June of the present year I made a trip to north Georgia, a region that has long been noted as one in which the inhabitants are very pale and anæmic, this condition being commonly reputed to be the result of dirt eating. Here I saw many instances of what was in all probability ankylostomiasis; but as a result of the ignorance of the people and their suspicion of all strangers a proper examination could be obtained in only four cases, in all of which the parasite was demonstrated. Subsequently a case of the disease was seen that originated in middle Alabama. During September and October I have been studying malaria in south Georgia and Florida, a region in which the people show profound anæmia even more often than in north Georgia. This condition is commonly ascribed to malaria, but my observations show that in almost all instances the sufferers have no malarial parasites in their blood, but eggs of the ankylostoma are constantly found in the feces. During my entire stay in this region I only saw one case of profound anæmia from malaria, and in this instance the patient did not exhibit the extraordinary anæmia so commonly found in those infected with the ankylostoma. I feel no hesitation in saying that time will show that by far the greater number of cases of anæmia in Georgia, Alabama, and Florida are due not to malaria but to the ankylostoma, and that this is the most common of all the serious diseases in this

region. There can be no reasonable doubt that what is true as regards the States named likewise holds good for the entire South. Since my first case was reported 13 other instances of the disease have been seen—11 originating in this State, and 1 each in Florida and Alabama; and if all of those encountered who were suffering from anemia could have been examined there can be no doubt that the number would be many fold greater.

"This communication is written in the hope that Southern physicians will take up this most important matter at once, for in no other serious disease does the victim suffer so long, in no other condition is he for such a period a menace to those about him, and in no other malady of such gravity is the treatment so rapidly and surely successful."

ITINERARY OF TRIP THROUGH THE SOUTHERN ATLANTIC STATES.

DISTRICT OF COLUMBIA.

In September, 1902, with the aid of three assistants (Messrs. P. E. Garrison, B. H. Ransom, and E. C. Stevenson) I began a systematic study of animal parasites among the patients of Government Hospital for the Insane, District of Columbia. From September 10 to December 12, 1902, the stools of 500 male patients were examined microscopically and 15 patients, or 3 per cent, were found to be infected with hookworms. The history has not yet been examined for each case, but probably most of the patients became infected in Cuba, Porto Rico, or the Philippines.

VIRGINIA.

Richmond and State farm.—Starting on my field work, my first stop (September 25) was Richmond, Va. Through the courtesy and cooperation of Dr. Charles V. Carrington I was able to examine at the State penitentiary and the State farm nearly 1,200 convicts. The prisoners filed past Dr. Carrington and myself in single file, and we selected those who appeared anemic or debilitated. Those selected were sent to the hospital and kept there until specimens of feces were obtained. In microscopic examination of 6 white male convicts no case of uncinariasis was found. The only zooparasitic case noticed was an exceedingly heavy infection with whipworms (*Trichuris trichiura*) in a man about 70 years old.

In 22 male negroes the examination was likewise negative so far as *Uncinaria* was concerned, but one case of infection of eelworms (*Ascaris lumbricoides*) was found. In several of the convicts starch digestion or meat digestion was poor, but in most instances the debilitated condition was due to tuberculosis or other diseases.

At the almshouse in Richmond 1 white female 28 years of age gave negative results; a weak-minded girl of 7 years showed a heavy infection with whip worms (*Trichuris trichiura*). In two negroes (1 male, 28 years; 1 female, 30 years) the examination was negative.

^a In 350 female patients examined to March 1, 1903, only one case of hookworm infection has been found.

It proved to be so difficult to obtain specimens of feces from the brickyards that no microscopic examinations were made. No clew was obtained to any disease among the workers which could be interpreted as probable uncinariasis.

Besides Dr. Carrington, I am indebted to Dr. Staton also for cooperation in my work at Richmond.

NORTH CAROLINA.

Virgilina Copper Mine district, southern Virginia and northern North Carolina.—Proceeding to the Virgilina copper mine district I found it very difficult at first to obtain specimens for examination. At one mine 1 white male and 3 negro males were examined; all were negative except 1 negro 22 years old, who showed infection with *Ascaris lumbricoides*. At this mine defecation under ground is prohibited; it occurs in the surrounding woods, at any place within a radius of about 50 meters from the shaft.

At a second mine three specimens of feces were taken at random from the woods; eggs of *Uncinaria americana* were found in one specimen, and ova of *Ascaris lumbricoides* in a second. The patients could not be traced. Specimens were then obtained from 4 white and 18 negro miners, the feces being taken without reference to the physical condition of the men. Of these 22 men, 1 negro 25 years old showed a light infection with *Uncinaria americana*, and inquiry developed the fact that he "had not been well for some time." His chief complaint had been "diarrhea." In two other cases, a white man 41 years old and a negro 34 years old, eggs of the eelworm (*Ascaris lumbricoides*) were found. At this mine defecation under ground is prohibited, and a box privy is located within about 50 meters of the shaft. The men prefer, however, to defecate in the surrounding woods.

I am indebted to Drs. F. D. Drewry and P. P. Causey, and to Mr. L. N. White, manager of the Person Consolidated Copper and Gold Mines Company at Durgy, N. C., for their kind cooperation in connection with my work in Virgilina and vicinity.

Cumnock Coal Mines, Chatham County.—The miners at this place were so suspicious regarding my work that it was almost impossible to obtain specimens for examination. In fact only two specimens could be obtained from about 40 men. One of these, a white miner, 52 years of age, and in rather anemic condition, showed infection with *Uncinaria americana*. The other, a white engineer, 34 years old, gave negative results.

Sanford, Moore County.—Specimens from 4 whites (2 males, and 2 females), obtained by 2 of the local physicians, were examined with negative results.

SOUTH CAROLINA.

Camden, Camden County.—Unexpectedly delayed at Camden, I visited the brickyards with Dr. J. W. Corbett. Of 7 specimens of feces picked up at random from the ground, probably most if not all from negroes, 1 was found infected with *Uncinaria americana*. One white laborer also showed infection with the same parasite.

Haile Goldmine, Lancaster County.—Through the kindness of Captain Thiess, the superintendent of the mines, and Dr. Gregory, the local physician, I was able to examine specimens from 5 white men and 5 negroes connected with the mine. All of these examinations were negative.

Upon leaving Richmond I happened to recall the observation made by Lucas (in Jordan & Clark, 1898, p. 70) in connection with uncinariasis of the seal pups of Alaska, namely, that the infected animals were almost invariably found on the sand rookeries; I also recalled that I had observed severe outbreaks of uncinariasis in sheep and goats on more or less sandy pastures, and further that a severe outbreak of the same disease among dogs had once been reported to me as occurring in a sandy yard. Not recalling at the time any similar observation for uncinariasis in man, I determined to test the subject at the first opportunity, and from Richmond to Haile Goldmine I had diligently inquired of every physician I met, whether he found more anemia on sand, clay, or rock soil. Most physicians replied that they had paid no attention to the subject; one physician stated that he thought anemia was more common in sandy than in clay localities.

Through Captain Thiess I learned that the land near the mines was chiefly a granite sand. With Dr. Gregory, I drove about 4 miles into the sandy district in Lancaster County and found a family of 11 members, one of whom was an alleged "dirt-eater." The instant I saw these 11 persons I recalled Little's (1845) description of the dirt-eaters of Florida. (See Stiles, 1902b, p. 208.) A physical examination made it probable that we had before us 11 cases of uncinariasis, and a specimen of feces from one of the children gave the positive diagnosis of infection with *Uncinaria americana*. There were hundreds of eggs present.

Inquiring for the largest plantation of this sand district, I was directed to a place in Kershaw County, about 6 miles from Kershaw, and through the kindness of Dr. Twitty and the owner of the plantation, I was able to make the desired examinations.

There are about 60 white "hands" on this farm. Going to a field I found about 20 at work. These 20 persons, men, women, and children, corresponded in more or less detail to the description of the so-called dirt-eaters and resin-chewers. A physical examination

showed that they also corresponded to cases of uncinariasis. A family of 10 members was selected and examined carefully. Specimens of feces from 4 of them were examined microscopically and found to contain hundreds of eggs of *Uncinaria americana*. The owner of the plantation informed me that it would be a waste of my time to examine the remaining 40 "hands," as they were in exactly the same condition as the 20 already examined.

Driving to a neighboring farmhouse, I found a family of 5 members, 3 of whom presented such severe and typical symptoms that I had no hesitation in diagnosing them as due to uncinariasis.

Kershaw County.—While driving back to Kershaw, I passed a country schoolhouse. The children, about twenty-five or thirty in number, were at play during recess, and a mere glance at them was sufficient to show that 30 to 40 per cent presented the same general appearance as the children on the neighboring plantation.

At Kershaw several extreme cases were met on the street. The persons in question had come in from the country. One farmer, living about 9 miles away from Kershaw, had with him two of his children. He stated that his entire family, 10 in number, had suffered or were suffering in the same way as these two boys. Physical examination made uncinariasis probable, and the microscopic examination of the feces showed heavy infections with *Uncinaria americana*.

Inquiry among the local physicians and the more intelligent laity elicited the information that the cases that I had seen represented conditions which were usually attributed to "dirt-eating," "resin-chewing," "heart disease," "bloat," "amenorrhea," "anemia due to malaria" (mosquitoes were noticeable chiefly by their absence), "general debility," "poor nourishment," etc. I was further assured that these conditions were general throughout this region, and were not, or only slightly, amenable to treatment.

Taking these cases together, some forty or fifty in all, which I examined carefully within three days, we have one common symptom, namely, *anemia*; nearly all other symptoms noticed could be reduced to sequelæ of *anemia*; further, in every case examined microscopically, exceedingly heavy infections with *Uncinaria americana* were found. Under these conditions, and because the general clinical history corresponded so well with uncinariasis, I have not the slightest hesitation in grouping the cases observed as due to *Uncinaria americana*.

Charleston, Charleston County.—Through the kindness of Drs. John Dawson and Robert Wilson, jr., I was able to meet the students of the Charleston Medical College. Explaining the object of my trip, I asked for volunteers to submit to microscopic examination. Sixteen of the students and 1 member of the faculty immediately volunteered. Of these 17 men (all white, of course), 4 were found infected with *Uncinaria americana* and 1 showed a heavy infection with

Hymenolepis nana. The cases of uncinariasis came from the sand districts—namely, Barnwell County; Florence, Saint John County; a sea-coast island near Charleston, and Edisto Island, Charleston County.

Through the courtesy of Dr. Huger and the ladies in charge of the Charleston Orphan Asylum, I was able to examine 230 white children, both boys and girls. I picked out 20 for closer examination, because of the anemic condition, or stunted growth, etc. Of the fecal specimens obtained, 15 showed infection with *Uncinaria americana*. All of the children came from sandy districts of the State—namely, Summerville, Dorchester County; Berkeley County; Adams Run, Colleton County; Plum Island, Charleston County, and McClellanville, Charleston County. One additional case failed to show eggs in the feces, but the clinical history during early childhood seemed typical for uncinariasis (see p. 58, case of L. B.). Of the 4 remaining cases (2 from Charleston (city) and 2 from Edgeville), 1 Charleston boy 11½ years old and 1 Edgeville boy 11 years old showed infection with whip worms (*Trichuris trichiura*).

Besides the Charleston physicians mentioned above, I am indebted to Dr. Grange Simons, president of the State board of health; Dr. J. Mercier Green, city health officer, and several other gentlemen, for their kind cooperation in my work. Dr. De Saussure stated to me that he had found the eggs of *Uncinaria* in the stools of several patients.

GEORGIA.

Atlanta, Fulton County.—Learning incidentally that Drs. Claude A. Smith and H. F. Harris were continuing their studies on uncinariasis in Atlanta, I left the city without seeing any cases personally. No reason was apparent which made it necessary to confirm their work for this locality, and, furthermore, the territory belonged to them as local men who were carrying on their investigations at their own expense.

In connection with northern Georgia it may, however, be stated that according to Dr. Lamartine G. Hardman, a member of the Georgia legislature, a condition exists in Jackson County, Ga., which corresponds to what I found in Kershaw and Lancaster counties, S. C.

Macon, Bibb County.—Through the kindness of the local physicians I was able to examine two white orphan asylums in Macon.

In one of these, Dr. Clark (consulting physician) and I selected from among the 85 children 17 boys and girls for closer examination. The selection was made in the same manner as at Charleston, namely, because of the pale, weak, or otherwise poor condition. Microscopic examination showed 12 cases of infection with *Uncinaria americana*, 2 cases of infection with *Hymenolepis nana*, and 3 negative cases. The uncinariasis cases came from sandy districts, namely: Americus, Sumter County, 1; Buena Vista, Marion County, 2; Thomasville, Thomas

County, 1; Savannah, Chatham County, 1; and Waycross, Ware County, Ga., 1; and Wacissa, Jefferson County, 1; De Land, Volusia County, 4; and Liveoak, Suwanee County, Fla., 1. Both *Hymenolepis* cases came from Georgia.

At another orphan asylum, through the kindness of Dr. Little, the consulting physician, I examined 112 white children, all from Georgia. I selected 21 for closer study, and the microscopic examination of the feces revealed 17 cases of infection with *Uncinaria americana*, 1 light infection with *Hymenolepis nana*, and 2 negative cases, 1 of which was doubtless malarial. The 17 cases came from the following places: Baxley, Appling County, 1; Cordele, Dooly County, 1; Darien, McIntosh County, 1; Effingham County, 1; Johnson County, 1; Jones County, 4; Kinderlou Station, Lowndes County, 1; Lyon, Tattnall County, 1; Monroe County, 1; Richwood, Dooly County, 2; Sandersville, Washington County, 1; Waycross, Ware County, 2.

Four cotton mills were next inspected, some of the houses of the factory hands were visited, and about 25 or 30 cases of uncinariasis were found. Inquiry developed the important facts that the infected persons had come to the mills from the rural sand districts, and that in general their condition improved with their residence in the city. An examination of the premises, both of the mills and of the houses, did not indicate that the disease would spread.

At a negro school in Macon I failed to find a single case of uncinariasis which could be diagnosed symptomatically with even a semblance of confidence. In one case only did I even suspect the disease.

"Circus day" brought thousands of people into view on the streets, many coming in from the surrounding country. Several cases of probable uncinariasis were observed among the whites, but none among the negroes.

Besides the Macon physicians mentioned above in connection with the orphan asylums, I am under obligations to Dr. A. M. Burt, and especially to Dr. H. McHatton for their cooperation in my work.

Milledgeville, Baldwin County.—Through the kindness of Dr. T. O. Powell, superintendent, and Dr. M. L. Perry, pathologist, of the State sanitarium, I was able to see a large number of the patients. Extreme cases of anemia were conspicuous by their absence. Two patients were selected as possible cases of uncinariasis, the microscopic examination being left in the hands of Dr. Perry, who has kindly written me that it was negative.

Fort Valley, Houston County.—With the cooperation of Dr. M. S. Brown, a local physician, I found about 10 cases of uncinariasis near town within an hour's time. The clinical histories were so typical that it was considered scarcely necessary to make a microscopic examination; nevertheless this was done in one case with the result of finding a severe infection with *Uncinaria americana*. After seeing the class of cases I desired to find, Dr. Brown assured me that they

were common in that region, and that he could easily find 50 or more cases within a day's time.

Albany, Dougherty County.—Leaving the sand district I next visited Albany, which is surrounded by clay. Corresponding to the change in the soil there was a change in the medical facies. Uncinariasis disappeared, except for cases which came in from the neighboring sand districts, while malaria increased. Dr. Hilsman, one of the local physicians, kindly drove around with me to find cases, but we were obliged to go about 15 kilometers (9 miles), namely, into Lee County, before we located a family with uncinariasis. During this drive we left the clay soil and passed into a sand district, with pine woods. The family in question presented four typical extreme cases; although microscopic examination for sake of diagnosis seemed unnecessary it was nevertheless made and showed heavy infection with *Uncinaria americana*.

While with Dr. Hilsman in Albany I made a most fortunate mistake in diagnosis. The patient was a boy about 13 or 14 years old. He had a clear case of malaria (typical history, enlarged spleen, etc.), but in addition to that I was confident that he had a medium or light infection with uncinariasis. Microscopic examination proved me in error. This boy lived in a clay district and had never lived in sand; furthermore, he did not show the eye symptom, which I shall discuss later (see p. 65). This is the last time I attempted a definite diagnosis upon symptoms of any medium or light case unless severe cases occurred in the same family.

As soon as Dr. Hilsman understood the kind of cases I desired to see he stated, with the utmost positiveness, that they did not arise in and around Albany until the sandy soil was reached. Occasionally cases came to Albany from the neighboring sand counties, but the local anemia Dr. Hilsman considered to be almost entirely of malarial origin—an opinion in which I can only concur after what I saw in that city.

I happened to be in Albany on Saturday, when the country folks for miles around come to town. Standing on the street corner for several hours, I must have seen about 200 whites and fully 3,000 negroes who drove or walked into town. Of the whites, I noticed about 5 cases of probable uncinariasis. Two of the cases, whom I was able to interrogate, gave a typical history of uncinariasis extending back for several years, but no history of malaria. They came from neighboring sand counties. Among the 3,000 negro men, women, and children, whom I saw, there was only one person in whom I even suspected from his general appearance that uncinariasis might be present.

Willacoochee, Coffee County.—In southern Georgia, Coffee County bears the reputation of being more or less a center for dirt-eaters. It is a sandy, pine-wood district, with numerous swamps, which indicate a more impervious subsoil. As a result, both malaria and uncinariasis

were found, and it was exceedingly interesting to note the ease with which an error in diagnosis in medium and light cases could be made if the microscope were not used. Extreme cases, however, could be easily distinguished without the microscope. Several cases of typical extreme uncinariasis were seen on the street, but not examined carefully. Then, through the kindness of Dr. Wilcox, I was able to make a careful examination, both physical and microscopic, of a group of 8 cases at the sawmills a short distance from town. After Dr. Wilcox examined these cases with me, he declared that he knew of at least 200 similar patients within the territory of his practice. He considered this condition one of the most common diseases of that region, but thought malaria was fully as common if not more so.

Waycross, Ware County.—Passing now to Waycross, I entered a district where uncinariasis is exceedingly common. Drs. R. P. Izlar and J. L. Walker assured me that the cases I was tracing, two of which I saw with Dr. Walker, were much more common than was malaria, the proportion being about 20 to 1. In this district these patients are called "Branch-water people." Both Dr. Izlar and Dr. Walker stated that they could easily show me scores of cases within a radius of a few miles.

FLORIDA.

Jacksonville, Duval County.—In Jacksonville two cases of typical uncinariasis were seen on the street. One of these patients was traced to the county in which the boy had formerly lived.

Both the State and the city health offices assured me that the condition I was hunting was perfectly familiar to them—that it was prevalent throughout the State, especially in the "flat-woods district," and that Florida physicians interpreted it as an anemia due to malaria and improper diet.

Waldo, Alachua County.—Through the kindness of the local physician, Dr. J. W. Boring, I was able to examine two groups of typical cases of uncinariasis within a short distance from town. Dr. Boring assured me that this condition was exceeding common in Florida and was generally interpreted as an anemia due to malaria and improper diet.

Ocala, Marion County.—In Ocala I saw several typical cases of uncinariasis on the street, and, through the kindness of Dr. A. L. Izlar, I was able to examine 5 positive cases and 1 probable case more closely. Dr. Izlar confirmed the statements of the health offices in Jacksonville, Fla., relative to the frequency and interpretation of the disease.

From Ocala I returned (November 16) directly to Washington, D. C., and noticed several cases which presented the appearance I had found typical of uncinariasis, as the train stopped at various stations in Florida.

SYMPTOMATOLOGY OF UNCINARIASIS.^a

In connection with the symptoms, let us recall that uncinariasis is caused by hookworms about half an inch long which live in the small intestine for several meters below the stomach.

These worms fasten to the mucosa and suck blood. They lay numerous eggs, which can be found by a microscopic examination of the stools. The number of eggs in the feces and, in a general way also, the severity of the symptoms will vary with the number of parasites present and with the duration of the infection.

The injury to the patients results from the following factors: (1) Sucking of blood by the parasites, which is a constant drain on the system; (2) loss of blood into the intestine through the minute wounds made by the parasite, a factor which also tends to deplete the system; (3) the wounds form points of attack for bacteria, hence increase the chances of bacterial infection as well as of toxic infection from partly digested and decomposed food; (4) the wall of the duodenum and jejunum becomes thickened and degenerated, and its function is thus decidedly interfered with; (5) the parasites in all probability produce a poisonous substance which acts upon the patient.

Theoretically there is only one sign which is present in every case, namely, the presence of one or more parasites in the intestine. If

^aThis discussion of symptoms will be influenced to no slight degree by the fact that during my trip my associates have been almost entirely practicing physicians, particularly in rural districts, rather than laboratory specialists; and, since it is more particularly the country practitioner whom I desire to reach by this paper, I shall not hesitate to use vernacular names even if these do not invariably have a classical origin.

My trip was undertaken in order to prove the frequency and geographic distribution of the parasite, not to study the symptoms it causes. It was therefore a zoological, not a clinical, trip, and on this account a zoologist, not a clinician, undertook the investigation. Not posing in any sense of the term as a clinician, I feel that any observations which I have made upon symptoms, *sensu stricto*, should be looked upon as over and above the amount of work which should be justly expected of me. Certain symptoms I could not help noticing. The circumstances of my trip, the rapid travel, short stops, and the fact that the work was done among strangers, and usually in the field instead of in a hospital, absolutely excluded certain observations, even had I considered that I was the proper person to make them.

If, therefore, the reader misses in this discussion observations on any particular symptom in which he is especially interested, I beg that he will recall that it is self-understood that the finer points in symptomatology must be studied by expert clinicians.

I regret that it is not feasible for me at the present time to review the entire medical literature on uncinariasis. Such an undertaking would involve an unjustifiable delay in sending much-needed information to physicians in the infected district.

In connection with my own observations, however, I shall make frequent references to the noted paper entitled "Observations on 400 cases of anchylostomiasis," published in 1894 by F. M. Sandwith, M. D., physician to the Kasr-el-aini Hospital, Cairo, Egypt, thus supplementing my description with the views expressed by a trained clinician.

these worms are in an egg-laying stage ova will be found in the feces. But from a practical standpoint, severe cases present what seems to be a characteristic type, and even medium cases often present a more or less typical clinical history.

The statement is not infrequently made that there is one way and only one way to diagnose a case of uncinariasis, namely, by examination of the stools to find the parasites or their eggs.

Academically this statement is more or less correct, yet practically



FIG. 42.—A severe case of hookworm disease observed in Florida. Note the bloated face, the drooping shoulders, the prominent abdomen, and the thin arms and legs. This girl is about fifteen years old. Original, from a kodak photograph.

it should be somewhat modified. Sandwith (1894, p. 13), to quote from a clinician, states that “facies of the patient is characteristic, though it is difficult to describe his discontented, harassed expression, which sometimes changes to a ready smile after a month’s stay in a hospital.” The data at my disposal would lead me to divide the cases of hookworm disease I have observed into three general but not very sharply defined classes, namely:

(1) *Light cases*, including those in which practically no distinct symptoms of the disease are noticed, but in which a few hookworm

eggs are discovered in the stools. We may also place here a number of cases in which, in addition to the presence of eggs, there is a slight diarrhea or some other slight symptom, including more or less rapid exhaustion after physical exertion, hence an indisposition to work, which is usually interpreted as laziness. Cases of this class are found in the infected areas and elsewhere, since infected people may move away from the sand districts.

(2) *Medium cases*, including those in which the disease has progressed to such an extent that a more or less anemic condition is noticed, but other symptoms are not especially marked. If these patients were found outside the infected area, the diagnostician (especially if he is not familiar with the disease) would probably not see anything particularly characteristic in them; many of these cases, however, show a more or less typical history, and if a history of residence upon sandy soil in tropical or subtropical regions can be obtained, uncinariasis should certainly be strongly suspected. If these cases occur in a family which also presents severe cases of uncinariasis, the diagnosis of hookworm disease in the medium cases is usually quite safe, even if a microscopic examination is not undertaken.

(3) *Severe cases*, in which we find that striking set of symptoms which even the laity in our Southern States attributes to "dirt-eating." These patients present a facies which is well recognized by Southern physicians. If the patient is found in a Southern sand area, the diagnosis is practically certain. If found outside of the infected area, with a history of previous residence in a Southern sand district, its recognition symptomatically ought not to be attended with difficulty. In case of doubt, if a microscope is at hand, the test may be made in less than five minutes; if no microscope is at hand, the blotting paper test (see p. 81) will usually suffice.

Turning now to an analysis of symptoms, I will give my observations on the severe cases. It is needless to state that the symptoms discussed may vary in intensity, and that not every symptom mentioned is found in every severe case. We find on the contrary an imperceptible gradation between the severest and the lightest cases.

GENERAL PREDISPOSING FACTORS.

INFECTION OCCURS CHIEFLY ON SANDY SOIL.

In connection with the clinical history, the residence on sandy soil is undoubtedly one of the most important points to be obtained. If an anemic patient gives no history of temporary or permanent residence on a sandy soil, uncinariasis is not absolutely excluded, but according to my experience the chances are against it. If on the other hand a history of sojourn or residence in a sandy rural district is obtained, the probabilities of uncinariasis are decidedly increased.

Nearly every case of the disease found during the entire trip was either living at the time in a sandy district or had lived in such a place a few years previously. As soon as I entered the sandy areas, uncinariasis was found. As soon as I left the sand, as at Albany, local foci of infection of uncinariasis disappeared.

Inquiry among physicians at first failed to elicit any definite statement regarding the soil on which anemia was most frequent, but upon going farther south several physicians were met whose experience fully confirmed my observations on this point. One physician in particular, Dr. A. M. Burt, of Macon, was of the emphatic opinion that in bringing the condition which I have interpreted as uncinariasis into connection with the sandy soil, I had found the keynote to the distribution of the entire disease. Dr. McHatton, of Macon, called my attention to the fact that in antebellum days the slave owners in the Lower Mississippi Valley frequently provided special quarters, which were removed from the sand districts, and to which they sent the dirt-eating and other sick negroes and also negresses about to be confined, the view being held that a clay soil was more salubrious than a sandy soil. This view, in fact, I found to be rather prevalent among the farm hands. Time after time they remarked, "We were never sick so long as we lived in a clay district," "This disease developed after we moved upon sand," etc.

This view that uncinariasis follows the sand is supported by evidence obtained in Alaska by Mr. F. A. Lucas. After the discovery was made that uncinariasis, caused by *Uncinaria Lucasi*, was prevalent among the seal pups, Lucas (see above, p. 39) showed that it was practically only the seals on the sandy rookeries which were infested with the parasites.

As stated above (p. 39), I have also observed two outbreaks of uncinariasis among sheep and goats, caused by *Uncinaria trigonocephala* (Rudolphi, 1809) Ralliet, 1900, on more or less sandy soil, and have further confirmatory facts in connection with one outbreak of the disease among dogs. Since returning from my trip I have found at least one reference in literature on uncinariasis and ground itch to the effect that the soil in districts where certain cases have occurred was more or less sandy (see p. 62), but I have not yet found that any author lays stress upon this point.

In view of all the data at hand, I have no hesitation in expressing the opinion that uncinariasis, caused by *Uncinaria americana*, is pre-eminently a disease of sandy localities and that cases found in clay or rocky areas can usually be traced to a former visit or residence in a sandy place.

Just why this disease should follow the sand rather than the clay is not absolutely clear. Three explanations have occurred to me as working hypotheses which, though not absolutely satisfactory as final, will, I believe, explain part of the mystery.

(1) We know that uncinariasis is spread through the feces: we know further that when the embryos hatch from the eggs they leave the feces and enter the surrounding water or moist earth, while there is no satisfactory evidence to show that they are blown around in the air in a dry state. (See Stiles, 1902b, p. 199.) Now, assume that a person walks over infected ground; if that ground is clay, he does not disturb the embryos which have crawled beneath the surface, except in wet places; if, on the other hand, the ground is sand, he not only stirs it up while walking, thus bringing the young worms nearer the surface again and thereby increasing their chances of producing an infection, but he is also likely to carry away particles of sand, together with embryos, with him on his shoes or feet, thus increasing his chances of becoming infected. It is further clear that children playing in sand will stir up more embryos than when playing on a clay soil, and will thus increase their chances of infection.

(2) An additional explanation is that water will not pass through clay as it will through sand; hence on clay soil the embryos stand a greater chance of perishing or of being washed by rain into the streams. On sand, however, the embryos might perhaps work their way through the soil^a with the water, and thus infect surface wells. In advancing this hypothesis, I am not unmindful of the view, supported by excellent observers, that, since the embryos sink in water, drinking water is not necessarily a common source of infection. Granted that they do sink in water, a water bucket in a well also sinks, and the water from surface wells frequently contains sand particles that are heavier and larger than *Uncinaria* larvæ: hence we can not altogether ignore the drinking water as a possible source of infection. If, on the other hand, drinking water were the only source of infection, it is probable that in families where uncinariasis exists the intensity of the disease would show a greater tendency to uniformity.

Giles has examined 56 specimens of water from wells and ponds of villages affected with hookworm disease and 16 of these he found by chemical and microscopical examination to be "bad" or "very bad." Yet on only one occasion did he find a rhabdite of doubtful origin in water. (Sandwith, 1894, p. 9.)

(3) Oxygen is necessary to the development of the embryos and larvæ, and it does not seem unreasonable to assume that sand would

^a In an article which has just appeared, Looss (1903, p. 331) says:

"Further, during the six years of my residence in Cairo [Egypt], I have not heard, up to the present time, of a single case [of hookworm disease] in an European (my own case, of course, excepted). This fact speaks all the less [so much the less] in favor of the assumption of a more common dissemination [i. e., infection] of the disease through the drinking water, vegetables, etc., since [as] *the mature hookworm larvæ, as experiments have shown, pass through the ordinary sand filter with surprising rapidity, and this even when the water is allowed, not to run off, but to stand.*" [Italics not in the original German.]

on that account present more favorable conditions for the growth to the "encysted" stage, and probably also a longer preservation of that stage.

In this connection it may be noted that Looss has used charcoal as a medium in which to cultivate the larvæ of *Agchylostoma duodenale*.

INFECTION OCCURS CHIEFLY IN THE RURAL DISTRICTS.

Since the infecting agent of uncinariasis is spread through the feces, we may expect to find infection taking place (other things being equal) in localities where the fecal matter is not properly disposed of. Furthermore, we would not expect that paved streets or grass lawns would favor the development of the disease. We need not therefore expect local foci of infection to occur in cities and towns which have proper sewerage systems and in which the streets and walks are paved and the yards sodded; but we may expect to find local foci of infection in localities where box privies are used but not properly cared for, or where promiscuous defecation occurs in the woods, fields, mines, etc. This condition is in fact exactly what is found. While uncinariasis is not entirely absent from those premises on which the feces are properly disposed of, in cities like Washington, Richmond, Charleston, Macon, Jacksonville, and Ocala, such cases as are found can not be shown to have developed there; but probably in most every case they can be either probably or positively shown to have been contracted elsewhere. In a number of cases I have established this point with a probability, which for all practical purposes may be looked upon as a certainty. We may therefore exclude the greater portion of the inhabitants of sanitary districts of cities from consideration in connection with uncinariasis and may with confidence lay down the general rule that any anemia developing in them as result of local infection (namely infection at home) is much more likely to be due to malaria or other causes than to uncinariasis.

As we approach the outskirts of cities and towns and enter the rural districts, localities in which box privies are used but not always properly cared for, we meet with conditions which are more favorable to infection with the hookworm disease. Accordingly, in cases of anemia, especially in women and children, developing in such localities, uncinariasis must be taken into consideration as one of the possible causes.

Sandwith (1894) states that his patients came from all parts of Egypt except some of the seaport towns.

As we go into the country and visit the farms we not infrequently find a condition, in respect to the disposal of fecal discharge, which almost beggars description. Taking the rural districts visited during my recent trip as example, it is not an exaggeration to say that with the exception of the planters' premises, not over half of the country houses or huts of the sand regions have any privy at all; if there is

one present, it is rarely properly cared for; furthermore it is the exception rather than the rule that it is used. As a result uncinariasis is widespread, not because the country air is particularly favorable to its development, but simply because so little attention is paid to the proper disposal of the fecal discharges.

SYMPTOMS ARE MORE SEVERE IN SUMMER THAN IN WINTER.

It is almost universally conceded that the patients are in better condition in winter than in summer. According to testimony, the symptoms begin to increase in the spring and to decrease in the early winter. This periodicity will be noticed, of course, only in localities which are above the frost line, and it is easily explained when we take into consideration the biology of the parasites. Cold retards and heat hastens the development of the eggs and the embryos; a freezing temperature of 24 to 48 hours' duration, it is said, kills both eggs and embryos. Accordingly, after frost sets in in the fall, the patients will add less to the infection which is present in their bowels than they will during the summer. Some of the worms already in the intestine will be passed, thus decreasing the number of parasites present; the patient will accordingly lose less blood and will on this account feel somewhat better. As warm weather begins in the spring the free eggs and embryos will develop more rapidly and the infection will be increased. There will be more parasites in the intestine, hence symptoms will be augmented.

Some few patients, however, insist that they are better in summer than in winter.

It is probable that the seasonal periodicity of the symptoms noticed in our Southern States will be modified in the Tropics, so that the symptoms will increase in severity in the rainy season and decrease in the dry period of the year. Such a periodicity would correspond to the biologic fact that the eggs and embryos perish very quickly upon becoming dry.

In patients who are not subject to continued infection, as for instance those who have left the area of infection, the seasonal periodicity may be expected to disappear.

WHITES APPEAR TO BE MORE SEVERELY AFFECTED THAN NEGROES.

Osler and other observers have already noticed that chlorosis is more frequent in blondes than in brunettes.

Uncinariasis occurs in both blondes and brunettes, and in both the white and the negro, but so far as my observations go the disease is more severe, *or at least more noticeable*, in blondes than in brunettes, and much more severe, as a rule, in the white than in the negro. This observation was supported by all the evidence I could gather from local physicians. In fact, several practitioners declared that they had never seen a case in the negro to recognize it. There is, however, abundant evidence that such cases do occur.

I am at a loss for a satisfactory explanation of the comparative freedom from uncinariasis noticed in the negro. They live under the same conditions as the poorer classes of the white population, except that, as a rule, the negro farm labor is more common in the rich than in the sand districts; however, negroes also live in infected districts. It is true, as frequently claimed, that some of the negro habitations are more clean than some of the homes of the poorer whites. Still, not all negro huts are cleaner than all white huts. The personal habits of the negro children are certainly no more hygienic than those of the white boys and girls.

One factor which may possibly play a rôle in this comparative freedom from the disease on the part of the negro is the fact that negro women very frequently give their children "worm tea," made from certain plants, in order to expel the "eelworm" (*Ascaris lumbricoides*), or they give calomel "to regulate the liver," and this may perhaps also result in expelling the hookworms before the latter have had an opportunity to do much harm. Still this explanation is not altogether satisfactory, especially in view of the testimony of both the local physicians and the negroes themselves to the effect that eelworms are much less common now than formerly, hence "worm tea" is not taken so frequently as in former years.

Chabert (1821a), Jordan (1832), Imray (1843), Le Conte (1845), Duncan (1850), and other authors report "dirt-eating" among the negroes. In fact, nearly all early authors who describe "dirt-eating" lay special stress on the frequency of the habit among the slaves.

In conversation with a negro druggist, I was informed that while dirt-eating was formerly said to be more or less prevalent among the negroes, it was an acknowledged fact among them that the custom had greatly decreased in recent years. In connection with this statement we may note the interesting claim by Cotting (1836a) that there was a reduction in dropsy and dirt-eating corresponding to the more general use of calomel.

Sandwith remarks upon the apparent comparative freedom of the negro from uncinariasis and anemia. Zinn and Jacoby (1896) also refer to the frequent presence of *Agchylostoma duodenale* in negroes of Africa, in whom the anemia was not prominent.

Can it be that the poison produced by the hookworms has less effect upon negroes than on whites and that on this account the disease is less severe in the dark races?

OCCUPATION OF PATIENT.

It is generally acknowledged by writers on uncinariasis that the disease is especially prevalent among people who in their daily work come in contact with earth. In fact, the malady is sometimes called "brickmakers' anemia" or "miners' anemia." Sandwith mentions "peasants" as apparently forming the majority of his patients.

In my own work I was surprised to find that hookworm disease was comparatively light and comparatively rare among the miners I examined. Of brickmakers my statistics are too small to permit any generalization. Two examinations out of eight showed light infections. Most of my cases were from the farming classes.

Sandwith emphasizes the fact that his patients came from the poorest class of the community, and that of 200 men 190 were accustomed to work with their hands in more or less damp earth. Of these 190 men, 152 were agricultural laborers; 18 were masons or bricklayers' laborers; 7 were "scavengers of street refuse and of cesspools, accustomed to emptying with their hands the dry contents of the latter;" 7 were peddlers of unwashed vegetables; 3 limestone carters and scavengers; 1 gardener; 1 fisherman in the mud; 1 "shadow" worker at the Nile bank; 1 coffee-stall keeper; 3 readers of the Koran; 1 blacksmith; 1 shoeblack; 4 beggars. Sandwith also mentions 20 cases among policemen, who dated their illness from periods of life when working as agricultural laborers.

SEVERE CASES ARE MORE COMMON IN WOMEN AND CHILDREN THAN IN MEN OVER TWENTY-FIVE YEARS OF AGE.

The assertion is frequently made that uncinariasis is more common in men than in women and children. This statement may be perfectly correct in mines and brickyards, but it does not hold good for the farming localities I visited, where the greater prevalence of severe cases in children than in adults, and in women and children than in men over 25 years of age, is very striking. The conditions found, in respect to this point, seem to be due to four factors in particular, namely: (1) The average family in the country districts numbers from, say, 6 to 12. As a rule, 2 or 3 of these can be called adults, and 3 to 9 can usually be classed as children (including minor boys and girls). Since there is a greater number of children than adults subject to infection, we should naturally expect to find a greater number of cases among the children, and our expectations are fully realized. (2) The fact that children and women present a greater number of cases than do the adult males over 25 years of age I am inclined also to explain on the ground that the former are at home more than are the men; the area immediately surrounding the house, for, say, a radius of 50 meters, is a more common place for defecation than are the more distant fields, hence it is more severely infested with the infecting agent. Now, while it is true that, among certain classes, both women and children work in the fields, it is also true that they do this much less than the men. They are at home more, therefore they are on the more intensely infected area for a greater length of time; hence, in respect to actual time they are more subject to infection than are the men, and, other things being equal, they will present a greater num-

ber and a greater proportion of extreme cases. (3) The children in playing in the sand around the house, and owing to their more careless personal habits, are of course especially liable to infection. (4) The men being stronger, are, as a rule, better able to withstand the effects of infection. An interesting and important fact is that men of about 20 to 24 seem in many cases to more or less outgrow the effects of the malady. They give a history of medium or severe infection from, say, 10 to 18 years of age, then at 18 or 20 they begin to improve, and finally appear much better, although their features and physique still show the effects of former disease. This time of improvement corresponds to the years following their first more active participation in work which takes them more away from the house, hence to years when they are subjected less constantly to infection.

Sandwith states that nearly all of his 402 patients were in the prime of life, between 20 and 40 years of age. His youngest case was a boy of 6 years; 48 patients were between 10 and 20 years of age; 170 patients were between 20 and 30 years of age; 140 were between 30 and 40 years of age; 21 were between 40 and 50; 15 were between 50 and 60; and 7 were above 60.

Giles reports a case of a girl 4 years of age. My youngest patient was 3 years old.

Of Sandwith's 402 cases only 3 were females. This statement is in striking contrast to my observations. Sandwith and I made our studies, however, under totally different conditions, for his patients came to his hospital, while I went to the homes of the infected persons. Possibly this will explain, in part, our different results, for he states that "women have not yet learned to apply for hospital relief in the same proportion as the men."

SEVERAL CASES ARE LIKELY TO OCCUR IN THE SAME FAMILY.

Uncinariasis commonly occurs in groups of cases. If one child in a family is accused of being a "dirt-eater," and is shown by physical and microscopic examination to represent an extreme case of uncinariasis, an examination of the remaining members of the family will usually show that most or all of them are suffering from anemia in different degrees, and the microscope will disclose infections with *Uncinaria*. This condition of affairs is so general that it may be looked upon as the rule among farming classes, while the occurrence of isolated cases in a family, except possibly among miners, may be looked upon as the exception. Numerous observations could be cited in support of this view; for instance, in the first family examined in the sand district near Haile Goldmine only one member had the reputation of being a "dirt-eater," but all 11 were in different stages of anemia. In a family of 10 members on a neighboring plantation only 1 was noted as a resin-chewer, but all 10 were anemic. In orphan

asylums it was noticed that if two or more children of one family were present and one child was affected, the other children were also affected. In fact, in every instance where I was able to examine the family to which an alleged "dirt-eater" or "resin chewer" belonged, all or nearly all the members of the family showed an anemia with the general history of uncinariasis, and in every case examined microscopically the eggs were found.

Two families may, however, live very close together, and one family may show several severe cases while the other may not show a single case severe enough to be suspected symptomatically. Thus, on one plantation all the hands examined showed uncinariasis, while the planter's family, of much more cleanly personal habits, did not exhibit any signs of infection. It is, however, more common to find that where one family is affected other families of the same neighborhood will show infection, provided of course that the infected family in question has not moved into a city or a clay district.

The occurrence of cases of uncinariasis of different degrees of intensity in family or neighborhood groups is easily explained by the fact that in a given family or neighborhood all persons are subject to the same general conditions of infection, but owing to differences in age, daily occupation, personal habits, etc., some will be more subject to infection than others.

OBJECTIVE AND SUBJECTIVE SYMPTOMS: ANALYSIS OF SYMPTOMS.

It is difficult to draw a distinct line between the subjective and the objective symptoms. If a patient is left to tell his own story, practically all that the observer will learn is that the patient "feels weak, has a headache, gets dizzy, has fluttering of the heart, finds it hard to breathe, feels worse in summer than in winter, and has 'misery' in the 'stomach.'" In medium cases a few judicious questions, directed more to the parents than to the patient, will as a rule bring to light a history upon which, taken in connection with what one can himself observe, a probable diagnosis may be made. As a rule, little weight can be placed upon the statements made by a patient suffering from an severe infection of uncinariasis. He will answer "yes" or "no" in a most contradictory manner, so that by putting questions in different ways it is practically possible to make him admit or deny, as desired, any particular symptom. It is chiefly from the patient's family that one must judge of what the person has complained.

ONSET AND INCUBATION.

As it takes the parasites four to six weeks to reach maturity after entering the system, the earlier symptoms will be more particularly gastro-intestinal (see, however, p. 60), and even if these are present in a marked degree we can not look for a diagnosis by microscopic

examination of the feces until the worms begin to lay eggs. It is, however, not excluded that some of the young worms might be passed in the stools and be identified, though such a chance is probably more theoretical than practical. In an experimental case of infection through the skin, Looss showed that eggs did not appear in the feces until 71 days after infection. According to Sandwith (1894, p. 12). Surgeon-Major Giles suspected that many of his patients in Assam had suffered from fever at the onset of their malady, and he was confirmed in this impression by observing pyrexia in the monkeys fed on hook-worm embryos.

Looss (1897, pp. 914-915) noticed nausea as the first symptom in dogs to which he fed the larvæ of *Agchylostoma duodenale*.

Bentley (1902 a) practically advances the view that "ground itch" is the primary symptom, and since Looss's view of infection through the skin is correct, at least for some cases, some cutaneous symptom must in such instances be the first symptom of uncinariasis. (See p. 60.)

STAGES OF UNCINARIASIS.

Since my observations of each patient were confined to one or two days, they would not justify me in dividing the disease into stages. Lutz (translated by Macdonald) recognized the following stages:

I. STAGE OF PURELY LOCAL SYMPTOMS.

(a) *Acute form*; (b) *chronic form*.—The symptoms are similar in both forms. The disease is yet limited to pains and disordered digestion; no pallor, no rise of pulse.

II. STAGE OF SIMPLE ANEMIA OR OLIGOCYTHEMIA (CHLOROTIC STAGE).

(a) *Acute form*.—1. Slight degree: Conjunctival vessels still visible; nails and lips pale red; pulse increased in frequency; no blowing murmurs over cardiac area.

2. Higher degree: Conjunctivæ devoid of vessels; nails whitish; lips pale; pulse frequently very much increased; no blowing murmurs.

(b) *Chronic form*.—Anemia has not reached the highest degree. In many cases distinct cardiac hypertrophy and dilatation; in other cases disordered valve closure; seldom both combined. Moderate increase of frequency of pulse.

III. DROPSICAL STAGE.

(a) *Acute form*.—A high degree of anemia: pulse small, much increased in frequency; no blowing murmurs; edema of a hydremic character.

(b) *Chronic form*.—Symptoms of cardiac defects, with disturbed compensation, or of fatty degeneration; distinct symptoms of cyanosis; dropsy of engorgement; anemia of varying intensity; disordered nutrition.

DURATION OF UNCINARIASIS.

In speaking of the duration of uncinariasis, we should clearly distinguish between the duration of cases which remain in the infected areas, and the duration of cases which after once becoming infected move to uninfested districts where conditions are such that reinfection is excluded.

In the former instance we have to deal with cumulative infection, taking place week after week and year after year, hence successive generations of parasites come into consideration.

In the latter instance we have to deal with the individual life of the parasites which are present in the patient at the time he leaves the infested area.

A failure on the part of most observers to distinguish between these two classes of cases renders the published data less valuable than they otherwise would be in determining the duration of uncinariasis.

In my own observations, I have found people remaining in the infested areas who gave a history of the disease extending back for ten, twelve, and even fifteen years. I have further three observations which I consider free from criticism in connection with patients who presented the disease six years (2 cases) and even six years and seven months (1 case) after being removed from the source of infection. I also have one observation, which is not free from criticism, upon a patient who showed the disease ten years after entering a hospital.

Sandwith (1894, p. 15) in discussing the duration of illness says—

“Nearly every patient said he had been ill two or three years before admission. I find among my notes 29 cases, uncomplicated by other diseases, where the patients said they had suffered more than three years. Of these, 13 had been ill for four years, 6 for five years, 3 for six years, 2 for eight years, 2 for nine years, 2 for eleven years, and one vowed it was fifteen years since the commencement of his symptoms.

“It may be safely said that during these long intervals of time very few patients had any rational treatment.”

It is probable that Sandwith's cases were subject to cumulative infections.

LENGTH OF LIFE OF THE INDIVIDUAL PARASITES.

It is already established that for every hookworm which is present in the intestine a separate embryo must enter the body. In other words, the eggs which the female worm deposits in the intestine will not develop there to mature parasites, but must first be discharged in the feces and undergo certain changes.

It is further clear that direct autoinfection, such as takes place in the case of pinworms (*Oxyuris vermicularis*), is excluded. For instance, suppose a child is at stool and soils his fingers with the feces, which contain hookworm eggs, then puts his fingers into his mouth and swallows the eggs; these ova will not develop in the bowels into adult worms.

Whether an *Uncinaria* egg, which happened to get under the finger nails, could reach the larval infecting stage in that place is perhaps an open question. So far as I am aware, the point has never been studied, but what is already known about this group of parasites does not lead me to believe that such a condition would be especially common, although it does not seem absolutely impossible.

With the foregoing premises in mind, it is important to determine how long the parasite in the intestine can live. Regarding the American hookworm, *Uncinaria americana*, I can present the following data: Of children at the Charleston Orphan Asylum in whom I demonstrated the presence of *Uncinaria americana* microscopically, it may be noted that 8 children had been in the orphan asylum two years or less; 4 children had been in the asylum between two and three years; 2 children had been in the asylum six years; 1 child had been in the asylum six years and seven months.

I have selected the Charleston Orphan Asylum as best fulfilling the conditions desired to illustrate the point at issue. The refined discipline, the scrupulous cleanliness, and the general hygienic conditions noticed are such that local infection is practically excluded. It must be admitted that some persons coming in from the country might possibly bring on their shoes a few embryos, but such a theoretical possibility is altogether too remote to explain the 15 cases found.

For all practical purposes we are justified in assuming that the hookworms which these 15 children had in them when I saw them were the same individual worms which were in the children when the latter entered the asylum, and from the data obtained it is clear that hookworms of the species *Uncinaria americana* are capable of living six years and even six years and seven months.

A sixteenth child (case of L. B.) examined showed a clear clinical history of uncinariasis of long standing. Her condition at the time of entering the institution, as described to me by Dr. Huger, and her present complexion, eyes, stunted growth, and inferior mental development leave no practical doubt in my mind regarding the diagnosis. No normal eggs were found in her stools, despite the fact that I made 25 slides. One slide showed a single abnormal egg which had evidently been dried and had clung to the slide after an examination in some other town. I do not know this girl's complete therapeutic history during the past twelve years, but from the absence of eggs in her stools it is necessary to conclude either that the worms had been expelled by the drugs taken or else *Uncinaria americana* is not able to live twelve years.

Ashford (see Stiles, 1902b, p. 210) mentions a case where a boy "had been the host of the worm for probably ten or fifteen years," but he does not state that during this time the patient was not exposed to further infection.

The clinical importance of the length of the life of the parasite is self-evident. Suppose a physician in the North has an anemic patient, or a physician in the South has an anemic patient who lives in the city or in a clay district; it is not sufficient to inquire whether he or she has recently been exposed to malaria, but inquiry should extend for eight or more years back in order to develop the fact whether she has

during this time visited any tropical or subtropical sand area. If such a fact does develop, uncinariasis is among the probabilities and a microscopic examination of the feces should be made.

GENERAL EXTERNAL APPEARANCE.

GENERAL LACK OF DEVELOPMENT—STUNTED GROWTH.

In severe cases of long standing the patient is undeveloped both physically and mentally. A boy or girl 12 to 14 years old may be as small as the average child of 6 or 8; a young man or woman of 18 to 22 years old may present the general development of a child 12 to 16 years of age, but the face may appear either like that of a child or like that of a very old person, especially like that of an elderly dwarf. Similar conditions have been described as a symptom of dirt-eating.

SKIN.

The skin has an anemic, waxy white to a yellow or tan, shriveled, parchment-like or tallow appearance. In general it is that color known in the South as a "Florida complexion." In some cases of malaria, if one trusts too much to the appearance of the skin, he is likely to be misled into an erroneous diagnosis of uncinariasis, but the general clinical history is usually sufficient to distinguish between the two diseases, while the microscopic examination gives a positive diagnosis. (See also, Temperature, p. 72.)

Wounds heal slowly.—Among the first severe cases of uncinariasis found, I noticed that several of the patients wore bandages. Suspecting the possibility of ground itch and recalling Looss's and Bentley's theories (see below), I immediately inquired into the history of the sores. According to the testimony of the patients the ulcers present had started as small wounds produced mechanically; the wounds had failed to heal promptly, had grown worse, and were now about a year old. This same story was related to me by quite a number of patients, and many other persons suffering from uncinariasis assured me that in their cases cuts and bruises healed very slowly, testimony which was repeatedly corroborated by local physicians.

Cutaneous lesions caused by uncinariasis.—The statements just made lead to a consideration of certain views recently advanced by Looss and Bentley.

Looss's theory of cutaneous infection.—Looss (1901) has shown that if a drop of water containing embryos of *Agelostoma duodenale* is placed upon the skin an itching sensation is produced; the worms enter the hair follicles, and from there they seem to bore into the surrounding tissues. Looss further advanced the rather startling opinion that the larvæ then reached the intestine, and he recounted observations which gave a certain amount of plausibility to this view.

More recently (see Sandwith, 1902). Looss has performed experiments which, so far as can be judged at present, demonstrate the correctness of his theory. According to Sandwith (1902). Looss^a smeared on the back of a puppy a mixture of charcoal and feces in which hookworm larvæ had been bred. Between nine and ten days afterward the puppy died and was found to have anemia of most of his organs, and a plentiful supply of young hookworms was found in his jejunum. A second puppy was treated in a similar way and also died on the night between the ninth and tenth days. Upon post-mortem he also showed exactly the same results. A man who offered himself for experiment was also similarly treated on his forearm, and in his case the first hookworm eggs were discovered in his feces on the seventy-first day. In all three experiments the feces were regularly examined for some weeks prior to the experiments, so that, so far as we can now judge, the results must be accepted, despite their very startling nature. Furthermore, Looss is known as too careful an investigator to permit any foreseen error to creep into his conclusion.

Somewhat similar results, namely the entrance of embryos into the skin, have been obtained by van Durme (1902, pp. 471-474) in experiments with *Strongyloides stercoralis* on guinea pigs, and it is needless to insist on the great importance of Looss's demonstration.

Bentley's theory of ground itch.—In line with Looss's views, Bentley (1902a) has made certain exceedingly important observations and experiments, the logical conclusion of which, taken in connection with Looss's work, seems to be that at least certain forms of ground itch constitute the initial symptom of uncinariasis. Bentley defines ground itch as follows:

Ground itch—Synonyms: *Panighao*, *water itch*, *water pox*, *water sores*, *sore feet of coolies*—is an affection of the skin, confined entirely to the lower extremities, and probably always associated with the presence of the larvæ of *Ankylostoma duodenale* in the soil of the affected areas; endemic in Assam and the West Indies and possibly present in other parts of the Tropics; characterized by its periodical epidemic appearance in the infected areas, coincident with the onset of the rainy season; with typical lesions consisting in a primary erythema, followed by vesicular eruption, which frequently becomes pustular and in severe cases may result in obstinate ulceration, or even gangrene.

Dr. Seheult (1900), of Trinidad, has suggested that the disease is probably due to some chemical irritant present in the soil, either natural or due to manure used in cultivation. Dr. Dalgetty (1901), of South Sylhet, struck by the resemblance which the lesions bear to scabies, and finding a mite present which he named *Rhizoglyphus parasiticus*, assumed this acarine to be the cause. These authors and Bentley (1902a) seem to agree that ground itch is a filth disease which

^a During the proof reading of the present report, Looss's (1903) article, detailing his brilliant experiments, has appeared. He demonstrates, beyond any question of a doubt, the correctness of his contention that infection with hookworms may take place through the skin.

is increased by the lack of care given to the proper disposal of alvine discharges.

Bentley (1902a) found in a water sore a young worm which he considered to be identical with *Agchylostoma duodenale*. He then performed the following experiments—

a. Some ordinary soil was sterilized by heat, and after being moistened with sterilized water, was infected with a small quantity of fecal matter containing numerous ova of *Agchylostoma duodenale*.

b. A similar preparation of soil was infected with a small quantity of feces, which on examination was found to be free from hookworm infection.

These two preparations were incubated at the ordinary temperature of the air for about a week, when sample “*a*” was found to be swarming with larval hookworms and various forms of bacteria and fungi, and sample “*b*” was similar in appearance except that no hookworm larvæ were found.

Each sample was then divided into two parts, *a'* and *a''* and *b'* and *b''*. Samples *a'* and *b'* were kept moist, while *a''* and *b''* were gently dried by exposure to the air for eight hours. Previous experiments had shown that six hours gentle drying at ordinary temperatures was sufficient to kill the hookworm larvæ. After remoistening *a''* and *b''* with sterilized water, the four samples were applied to the wrists of the subjects of experiment for eight to nine hours, and then they were removed. Fifteen hours after the first application, considerable erythema with a minute papular eruption appeared over the spot to which *a'* had been applied; within twenty-four hours a distinctly vesicular eruption had developed, followed by pustules exactly resembling those found in the lesions of ground itch. In the other cases a faint reddening of the skin was produced, which shortly afterwards disappeared. A reexamination of sample *a'* now showed that no live larvæ were present, although one or two dead worms were found. Sample *a''* still contained the dead larvæ. Apparently, therefore, the live larvæ *a'* had entered the skin and their entry had been followed by lesions similar to those found in water sore.

According to Bentley, also, it is probable that the acuteness of the inflammation attending an attack of the ground itch is largely governed by the nature of the organisms which accompany or follow the larval hookworms in their passage through the skin.

Regarding the treatment of ground itch, Bentley says that in the papular and early vesicular stage of the disease the application of a strong solution of salicylic acid in collodion or methylated spirit will cause the eruption to dry up, and so cut short the attack of the disease to one or two days. If, however, pus has formed, the only treatment of any service is the opening up and disinfection of the pustules with pure carbolic acid, silver nitrate, or nitric acid, and the after treatment of the sore as an ordinary ulcer. In cases attended with great swell-

ing, inflammation, and tendency toward the formation of sloughs, free skin incisions and the use of hot antiseptic footbaths are indicated.

Dalgetty (1901, p. 77) advises the application of a strong solution of lime and sulphur: strong phenyl solution is also beneficial, and a coating of coal-tar acts for a time as a preventative against infection; but when once the vesicles have formed pustules are sure to follow, and then the only remedy is to open them, evacuate the pus, and thoroughly cleanse them.

An anonymous writer (? Dr. Elliot, of Assam) in the *Journal of Tropical Medicine* (1900), gives the following directions:

"The indications are to get the case as soon as possible; to carefully cleanse the foot by soaking it in warm antiseptic solution; then open the vesicles with sharp pointed scissors, snip the loose skin away, and finally wash the parts with carbolic acid solution (1 in 40), and treat the resulting ulcer with carbolic acid, phenyle oil, extract of paroli leaf, zinc ointment, etc., according to circumstances. The soaking, washing, and dressing operations are repeated once or twice daily, and healing takes place in eight or nine days in favorable cases."

It will be noticed that Bentley does not definitely state that he adopts Looss's view of intestinal infection through the skin; nor does he definitely state that the hookworm larvæ act as anything more than carriers of bacteria. The conclusion would therefore seem to be that the ground itch with which he was dealing is a bacterial infection due very probably to fecal bacteria.^a If this interpretation is correct, its *dependency* upon uncinariasis does not seem to be proved, although its occurrence with the disease would seem to be established.

Additional facts (besides Bentley's experiments) which support the view that the ground itch, with which he was dealing, is more or less connected with hookworm infection are the following:

Ground itch occurs in the warm rainy season, especially in June, July, August, and September, and does not occur in cold weather, even when it rains: thus the seasonal distribution in general agrees with the infection period of uncinariasis. Grass-covered soil and smooth beaten roads do not cause it, neither does working in loose dry soil; and these conditions are unfavorable to the development of uncinariasis. The number of cases increases after a heavy rain and rapidly decreases during a hot spell, a fact which agrees with the biology of hookworms. "The soil itself is sandy, with clay here and there; a belt of pure sand, 40 to 60 feet thick, lies at a depth of 6 to 18 feet from the surface;" and uncinariasis is preeminently a sand disease.

Still the question is not quite so simple as would at first appear, and in connection with the subject the following points come up for consideration:

^aLooss has, however, proved that certain cutaneous symptoms follow the entrance of hookworm larvæ into the skin.

1. According to Bentley, ground itch is confined entirely to the lower extremities, and other authors state that it rarely extends above the ankles. According to the physicians in Georgia and Florida, it is found on other parts of the body also. I saw one case, said to be typical, where the disease was confined to one arm. Under these conditions is the panighao, discussed by Bentley, identical with the so-called "ground itch" which is so common in the southern portion of the United States, or is only a part of the American "ground itch" produced by hookworm larvæ?

2. If ground itch is the initial stage of cutaneous infection with uncinariasis (as Looss's and Bentley's views would seem to indicate), why should it be confined entirely to the lower extremities? If infection by uncinariasis frequently takes place through the skin, would not the hands and arms also, especially of children, and more particularly the soles of the feet and the palms of the hands, the spaces between the toes and fingers, and under the toe nails and finger nails, be the most common initial points of ground itch in case this latter is an initial symptom of uncinariasis?

3. On the same premises would we not commonly find lesions corresponding to ground itch on the abdomen of cattle, sheep, goats, dogs, cats, foxes, seals, and other animals suffering from uncinariasis? I will not deny that such lesions occur, but I have seen many cases of hookworm disease in certain of these animals and I have no recollection of having observed anything which corresponded to ground itch. If it were as prominent, in the animals named, as the typical "ground itch" of man which I saw in Georgia, I doubt whether I should have overlooked it. Possibly I did not have recent infections before me.

4. According to the testimony of Georgia and Florida physicians "ground itch" is exceedingly common; it occurs at some period in the life of practically every person, unless he lives exclusively in the city; it occurs in the healthy as well as in the sickly, and in persons who neither at the time of infection nor later show the slightest evidence of anemia. These statements, which I have repeatedly heard from Southern physicians, can not be said to indicate that Bentley's views are applicable to all cases of "ground itch" as we find this disease in this country.

5. "Ground itch" is said to be common in clay districts as well as in sand districts. Under these circumstances, why is uncinariasis so pre-eminently a disease of the sand areas?

6. Bentley states that "ground itch" is probably always associated with the presence of the larvæ of *Agchylostoma duodenale* in the soil of the affected areas. Doubts may, however, arise as to whether a sufficiently wide geographic range has been examined in connection with this point.

In view of the above considerations, it will be well to remain open

to conviction awaiting a more thorough demonstration of the broad application of Bentley's interesting and valuable views. But until better proof is advanced than has thus far been brought to my attention, I find it impossible to unreservedly adopt the opinion that American ground itch is *necessarily* connected with uncinariasis.

While not opposing the theory of infection through the skin, but admitting, on the contrary, that Looss has proved his point, I may state that the conditions which I saw in the southern portions of the United States do not indicate that any indirect method of intestinal infection is necessary in order to explain the severe cases of uncinariasis observed. The average boy or girl suffering from this disease is not conspicuous because of personal cleanliness. Bath tubs are not found in their homes, and from physical examinations I made I can testify that not only their hands and finger nails, but their entire bodies also, are far from a condition unfavorable to parasitism. Sucking the fingers, picking the teeth, biting the finger nails, or even eating a piece of bread with soiled hands will usually suffice to convey some dirt between the lips. The sand on which the children play must be heavily infested with hookworm larvæ, and it certainly can not be an exceptional occurrence that the children unconsciously carry microscopic worms to their mouth. Further, the chances for infection of surface wells, from which the drinking water is taken, are very great in any sandy soil. If, however, cutaneous infection were the rule, I should expect to find all barefooted children in the infested area suffering not only from ground itch the entire summer, but also from severe infections of hookworm disease.

Hair.—The hair on the head appears to be about normal, but in cases contracted before puberty, the beard and the hair on the body (pubis, armpits, arms, legs) are usually undeveloped. I have seen patients 20 years of age upon whose body hairs were almost absolutely lacking.

Breasts.—The breasts of females, who have contracted hookworm disease before puberty, remain more or less undeveloped. In a girl of 20 years of age, for instance, the breasts may not be developed beyond those of a girl of 8 or 9 years old.

Nails.—The color of the tissue directly under the nails varies with the anemia.

HEAD.

Face.—The face has an anxious, stupid expression, and in severe cases is more or less "bloated" (edematous).

In fact, a prominent symptom of uncinariasis in practically all animals in which it occurs is the development, in severe cases, of a more or less extreme edema. To use the rural vernacular, "the face bloats," and "the feet and ankles swell." The symptom in question is more or less irregular in man as it is in other animals, notably in sheep, appearing and disappearing at intervals. Upon several occa-

sions I was informed that this symptom interfered seriously with the school attendance, for if the children sat still a long time in school "they began to swell." Quite generally, as was to be expected, testimony was to the effect that the edema was less frequent in the winter than in the summer.

Eyelids; conjunctivæ.—An examination of the eyelids exhibits the visibility of the blood vessels in light cases, but an absolute marble whiteness in very severe cases, with all possible intermediate stages corresponding to the general degree of anemia.

Eyes.—While looking at the eyelids for anemia, the observer frequently notices that the pupils are dilated or that they dilate readily and that the eyes are dull, dry, and usually of a chalky white. If the patient is directed to stare intently into the observer's eyes, there will be noticed a symptom which it is difficult to describe, but which I have found more constant than almost any other noticed, namely: After a moment, the length of time *apparently* varying slightly according to the degree of the disease, the pupils dilate and the patient's eyes assume a dull, blank, almost stupid, fish-like or cadaveric stare, very similar to that noticed in cases of extreme alcoholic intoxication. I am not familiar enough with the stare of anemic patients in general to state how common this peculiar look is among them, nor have I found any of my medical friends who could give me much information on this subject; but I can state that among the several scores of anemic people whom I examined on this trip, in the severe cases with two exceptions, I found the eggs of *Uncinaria americana* in every one (whose feces were examined) in whom I observed that indescribable stare: the two exceptions in question were city boys, both of them sons of a confirmed inebriate; further I failed to find the eggs present in certain extremely anemic patients in whom the stare was not noticed. It certainly was absolutely absent from a number of typical cases of malaria. Toward the end of the trip, I found myself unconsciously relying more upon the presence or absence of the blank stare than upon any other single symptom, except of course the presence of the eggs in the stools.

I will not go to the extent of stating that this stare is diagnostic for uncinariasis (and I will even warn that in dark eyes it is less evident than in eyes of light color), for I do not feel that I have had experience enough with the peculiarities of eyes in various diseases to speak authoritatively upon the subject. I simply mention this peculiarity in connection with the discussion of the eye as a symptom which, as my investigations progressed, made more and more of an impression upon me. Upon calling the attention of several local physicians to this peculiar stare, they informed me that it was a totally new symptom to them, but that after examining several cases they found it a very prominent symptom. As a general rule the eyes in advanced stages are dry. In this connection it may be noticed that several

authors have mentioned the glassy appearance of the eyes of dirt-eaters.

Since my return, several of my clinical friends with whom I have discussed this symptom have expressed some skepticism in regard to the matter. Although thoroughly convinced of its existence, for I saw it too frequently to be deceived, I have written to several Southern clinicians requesting them to give me the benefit of their independent observations on this point. Up to the time of reading "galley proof" of this report the following replies have been received:

[Extract from a letter from Dr. Hilsman, January 2, 1903.]

"Replying to your letter, 24th ultimo, I have to say that I have examined the patients that we saw in the country, and on making them gaze intently at me as you directed, I observed the vacant stare that you described, but did not observe the dilation of the pupils. The stare is very much like that of an epileptic as he begins to recover from a fit.

"These cases have improved under treatment suggested by you. The little girl passed a large number of the worms."

[Extract from a letter from Dr. M. A. Clark, January 30, 1903.]

"I have delayed, hoping to find the eye symptom you mention, but I have not yet found it. My cases are improving slowly."

Dr. James Edward Stubbett, of New York, who spent some years in Central America and has seen many cases of hookworm disease, has stated to me that he has frequently observed the peculiar stare in the eyes of dirt-eaters.

In this connection it is also not uninteresting to note that some "worm doctors" claim to lay special stress upon the eye in making their diagnoses; also that dilation of the pupil, due to irritation by intestinal worms as well as to anemic conditions, is recognized by certain prominent writers on the eye; further, that dilation of the pupil is also a symptom upon which many children's nurses depend in suspecting the presence of worms.

Sandwith (1894, p. 12) states that the eyes of his patients showed a pearly white conjunctiva, singularly in contrast with the yellow color of the face. Dr. Scott examined several men for him with the ophthalmoscope, and found in half of them a normal fundus, and in the other half a very pallid fundus. The refraction tests showed astigmatism in many cases.

Nostrils.—The visible mucous membrane of the nostrils becomes pale in proportion to the anemia.

Lips and gums.—The lips also become pale in proportion to the anemia, the inner surface of the lips and the outer surface of the gums frequently presenting almost a chalky white appearance.

Teeth.—Irregularity of the teeth was so common among patients affected with uncinariasis that the question arose in my mind whether this was not to some extent an expression of the general underdevel-

opment of the body due to this disease. The point at issue calls for the opinion of a dentist rather than that of a zoologist. The decayed teeth of dirt-eaters have been recorded in early writings on this habit.

Tongue.—In some cases the tongue was coated. A number of observers have already called attention to this symptom, which was by no means general in the cases I observed.

Several authors have remarked upon the presence of black, brown, or purple spots on the tongue in cases of uncinariasis, and the view has been advanced that these present a valuable aid in diagnosis, and in some cases, at least, that they disappear on treatment. In the cases which came under my observation, I looked very carefully for this symptom. In many instances I found more or less distinct purplish to brownish spots, irregularly round or elongate in shape, and these may or may not be identical with the spots described by the authors cited. It was, however, noticeable that in many cases where these spots were observed, the patients were accustomed either to chewing tobacco or to dipping snuff. In fact, some of the spots in question I am inclined to refer to the use of tobacco or snuff. Whether all cases are to be explained in this way is, however, open to question, with probabilities against such explanation. Nevertheless, it is well for practitioners to be forewarned upon this chance of error in diagnosis. Several authors have assumed that the spots on the tongue represent a symptom which has only recently been observed in this disease. In this connection, it is interesting to note that early authors—for instance, Cragin (1836a) and Imray (1843)—in writing upon dirt-eating have described these same spots.

NECK.

The cervical pulsations are often very evident, and in some cases may be seen from 6 to 12 feet away. (See Circulatory system, p. 72.)

THORAX.

In emaciation the thorax corresponds to the general emaciation of the extremities. In some cases the ribs are very prominent.

Heart.—See page 72.

Breasts.—See page 64.

ABDOMEN.

“Pot-belly” or “buttermilk-belly,” dropsy, and tympanites.—The condition known as “pot-belly” or “buttermilk-belly” is exceedingly common in uncinariasis, especially in extreme cases in summer. It is a distension of the abdomen, due apparently to two factors: (1) a gaseous distension of the bowels, and (2) the presence of an excess of fluid in the abdominal cavity.

I hasten to add that I have not seen a single autopsy in man infected with uncinariasis, but the same abdominal distention is found in

animals suffering from this disease, and a number of post-mortem examinations, especially on sheep, have given the conditions described.

Lutz mentions flatulence of the lower abdomen as a common symptom, and Sandwith (1894, p. 11) found it present to a slight extent in one-third of the cases.

Hair.—See page 64.

Genitalia.—See page 78.

EXTREMITIES.

Nothing of any particular note was observed in connection with the bones; for the muscles see p. 73. In many cases the feet and ankles were swollen, and in several cases the legs were marked with ulcers. See p. 59.

According to Sandwith (1894, p. 12), edema of the legs, like albuminuria, is to be seen only in the worst cases, and some of these had general edema when admitted in a dying condition.

GENITALIA.

See page 78.

MUCOUS MEMBRANES.

All mucous membranes correspond, in respect to their color, to the grade of anemia. (See Eyelids, p. 65; nostrils, p. 66; lips and gums, p. 66; tongue, p. 67; genitalia, p. 78.)

EXCRETIONS AND SECRETIONS.

URINE.

I have no observations to record in regard to the urine. Sandwith (1894, p. 11) states that—

“The urine is not unlike that of ordinary anemia, neutral or alkaline in equal proportion, and rarely acid, pale colored, with specific gravity ranging from 1010 to 1015. A trace of albumen was present in all the most advanced cases, without casts under the microscope.”

According to Zinn and Jacoby (1898, p. 16), Lussana (1890) found in the urine of patients suffering from uncinariasis a poisonous substance of the nature of a ptomaine, which caused extensive changes in the rabbit's blood, especially a change in the number of red blood corpuscles, and also poikilocytosis. These results are said to have been confirmed by Arslan (1892).

FECES.

Consistency.—The feces may be hard or soft, according to the presence of constipation or diarrhea.

Reaction.—In reaction the feces may be acid, alkaline, or neutral.

Color.—In a large proportion of medium and severe cases, the feces are reddish to brown in color. In some cases blood is present.

Blotting paper test.—In about 8 out of 10 medium and severe cases, if a portion of the feces is placed upon white blotting paper, and allowed to remain there for twenty minutes to several hours, it leaves on the paper a reddish brown stain similar to a blood stain. This test will be found useful by physicians who are not prepared for microscopic tests.

Microscopic examination.—If feces less than twenty-four hours old are examined microscopically, the eggs will be found in various stages of segmentation. If feces over twenty-four hours old are examined the free embryos also are usually found. If free embryos are found in perfectly fresh feces, the diagnosis of infection with the worm (*Strongyloides stercoralis*) of Cochin-China diarrhea must be made, and this parasite we may find in the same patient in whom uncinariasis is present.

CIRCULATORY SYSTEM.

The symptoms of the circulatory system are the most marked and most common; they seem to develop after the symptoms of the digestive system, but before those of the nervous system.

ANEMIA.

In all medium and severe cases, the anemia is what first attracts attention. This varies in intensity not only in proportion to the degree of infection, but also to a considerable extent in proportion to the length of the period of infection. For instance, 100 worms may be expected to produce a greater anemia within a given time, say one year, than will 50 parasites. But 50 worms may be expected to produce more anemia in two years than in one.

In some medium cases, the blood vessels of the conjunctivæ may be more or less visible, and the visible mucous membranes of the nose, mouth, and vulva, may be more or less of a normal or subnormal color. In the extreme cases, these structures may be as white as marble or paper. In the same way the color of the skin will vary (see Skin, p. 59; Nails, p. 64) from an almost natural hue to a whitish, lemon yellow, or tan color.

"This worm is probably much more common in this country than supposed. The cases known to me to date are: Baltimore, Md.—1 case, 1 death; reported by Strong, 1901. Richmond, Va.—1 case, 0 death; reported by Thayer, 1901. Anne Arundel County, Md.—1 case, 0 death; reported by Thayer, 1901. Washington, D. C.—4 cases, 0 death; unpublished, Zool. Lab., U. S. Public Health and Marine-Hospital Service. Ohio—1 case, 0 death; unpublished, Dr. A. P. Ohlmacher (mentioned here by kind permission of the observer). San Francisco, Cal.—3 cases, ? deaths; unpublished, Dr. P. K. Brown (mentioned here by kind permission of the observer). Cuba—? cases, ? deaths; unpublished, Dr. John Guiteras (mentioned here by kind permission of the observer). Porto Rico—? cases; ? deaths; unpublished, Dr. P. K. Brown.

Blood.—I did not stop for blood counts, as these have been made by other men, and while they are exceedingly interesting from a pathological standpoint they have not appealed to me as so direct a method of diagnosing intestinal parasites as is the fecal examination. In general it may be said that the blood of man corresponds to the blood of sheep, goats, cattle, dogs, etc., suffering from the same disease; in other words, the severer and longer the infection, the thinner the blood. In the vernacular of the sandlapper, it is "like water." It may here be added that in early literature on dirt-eating, several authors remark upon the water-like appearance of the blood.

Speaking in more technical language, the blood has been shown by Ashford to possess the following characteristics:

"(1) A severe anemia, falling as low as that of Addison's anemia in count of red cells in some cases. (2) A very low hemoglobin average and a very low color index. (3) A marked eosinophilia in some cases; 40 per cent reached in one case. This follows the observation of Neusser. (4) No leucocytes common to the disease itself. Leucocytosis recorded is always apparently due to complications, as noted. (5) Frequent presence of normoblasts, and in some cases megaloblasts, but never a majority of megaloblasts. (6) Poikilocytosis common. Manson denies this."

Ashford gives the following interesting table of blood counts:

Date.	Current number.	Red cells.	Hæmoglobin.	White cells.	Percentage of polymorphonuclear leucocytes.	Percentage of small lymphocytes.	Percentage of large lymphocytes.	Percentage of eosinophiles.	Normoblasts per cubic millimeter.	Megakaryoblasts per cubic millimeter.	
November 4 ...	1	1,530,116	20	6,800	65	21	9	5	26	13	Poikilocytosis. Polychromatophilia, many microcytes and macrocytes. Treatment: Digitalis and peptomangan. Recount, November 23, 1899; Hæmoglobin, 16 per cent; red cells 1,880,000.
November 3 ...	2	697,776	20	7,960	59	17	15	9	144	46	Poikilocytosis. Polychromatophilia, many macrocytes and microcytes. Treatment: Pil. ferri, quin, et strychn., and Pil. arsenic. Recount, November 23, 1899; Hæmoglobin, 23.5 per cent; reds, 2,664,440.
November 4 ...	3	1,533,112	22	2,000	64	22	8	6	8	0	Poikilocytosis. No rouleaux. Treatment: Digitalis and peptomangan. Recount, November 23, 1899; Hæmoglobin, 23 per cent; reds, 1,973,328.
November 5 ...	4	1,200,000	15	4,200	64	23.4	6	6.6	109	8	Poikilocytosis. Polychromatophilia. Treatment: Digitalis, simp., ferri iodidi. Recount, November 23, 1899; Hæmoglobin, 17.5 per cent; reds, 801,104.
November 6 ...	5	1,484,440	10	6,000	64	24	8	4	12	0	Poikilocytosis. Slight tendency to rouleaux formation; macrocytes and microcytes, latter predominate over former. Recount, November 23, 1899; Hæmoglobin, 14 per cent; reds, 687,776.
Do.	6	2,193,328	23	8,800	65	26	8	14	123	35	Poikilocytosis. No tendency to rouleaux formation. Treatment: Digitalis, Pil. ferri, quin, et strychn.
November 7 ...	7	1,623,328	17	5,600	60	23	5	12	11	0	Poikilocytosis. Many macrocytes and microcytes. Slight rouleaux formation; marked chromotophilia. Treatment: Digitalis, liq. potas, arsenitis in increasing doses to gtt. 5 t. i. d.
Do.	8	2,064,664	23	4,800	74	17	6	3	28	0	Poikilocytosis. Rouleaux formation good. Treatment: Pil. ferri, quin, et strychn., and digitalis. Recount, November 23, 1899; Hæmoglobin, 31 per cent; reds, 3,084,440.
Do.	9	1,271,104	14	7,800	60	17	6	17	30	15	Has elephantiasis anabum, but I can not identify the filaria as yet. Microcytes predominate over macrocytes. Good rouleaux formation. Treatment: Peptomangan. Recount, November 23, 1899; Hæmoglobin, 24 per cent; reds, 2,529,000.
Do.	10	1,600,000	17	1,500	72	20	4	4	6	0	Poikilocytosis. Recount, November 23, 1899; Hæmoglobin, 13 per cent; reds, 608,888.
November 8 ...	11	1,800,000	25	4,600	69	22	7	2	9	0	Poikilocytosis.
Do.	12	2,266,686	30	7,680	52	26	12	10	0	0	Poikilocytosis. Rouleaux formation good.
November 9 ...	13	1,268,888	20	6,800	65	17	7	13	0	0	Poikilocytosis. Rouleaux formation slight; has abscess of the liver.
Do.	14	2,410,000	25	11,000	50	10	9	31	0	0	Poikilocytosis. Rouleaux.
Do.	15	2,333,328	17	12,700	73	17	4	6	0	0	Poikilocytosis. Rouleaux.
Do.	16	2,924,444		5,200	72	20	5	3	10	10	Tertian malarial parasites found. Rouleaux good.
November 10 ...	17	2,140,000	23	18,000	40	12	8	40	36	0	Poikilocytosis. Rouleaux good. I believe this man to have been suffering from pneumonia at the time of examination, although the differential seems strange.
November 20 ...	18	3,524,440	55	11,800	88	9	3	1.5	0	0	Only case not presenting ankylostomes. Has tuberculosis.
November 12 ...	19		30	9,000	60	16	10	10	125		Poikilocytosis. Rouleaux formation good.
November 20 ...	20	1,620,000	16	2,400	72	10	5	13	0	0	

a Unknown.

Cervical pulsations.—In the rural vernacular, “jerking at the neck” or “jumping at the neck” refers to an anemic symptom which is exceedingly prominent in most medium cases and in all extreme cases of uncinariasis. It is simply the violent pulsations of the cervical vessels, visible sometimes at a distance of 2 to 4 meters.

Heart.—Nearly all medium and severe cases complain of having “heart disease” or a “fluttering of the heart,” and many of the patients are taking medicine for this symptom. (See also p. 35.) We have here, of course, the usual cardiac symptoms of an extreme anemia.

“Palpitation over the heart, in the epigastrium, and in the temporal arteries is sure to be present in bad cases, while the anemic murmurs of heart and neck are solely dependent upon the degree of anemia, and can be banished by a prolonged course of iron. Hypertrophy of heart was noted and verified after death in some of the advanced cases.” (Sandwith, 1894, p. 12.)

Pulse.—Pulse varies from 80 to 132 per minute. In medium and severe cases I noticed about 120 per minute probably more frequently than either a higher or a lower pulse. This was found in young and middle-aged (probably more commonly in children), in males and females, and yet without a temperature which was distinguishable by the hand as especially high.

TEMPERATURE.

Not being able to follow any cases for any length of time, hence not being able to make continued observations on the temperature, I considered that observations in other lines were more important under the circumstances. Hence I did not take temperatures carefully. According to observations by various clinicians, there may be subnormal or normal temperature, or the thermometer may register 100° to 102° F.

“The skin is always cold, and the temperature before thymol generally subnormal in uncomplicated cases. After excluding any fever produced by concurrent diseases and any defervescence caused by thymol, I find that one-third of my patients had a normal temperature during their stay in the hospital, but that two-thirds had a distinctly subnormal range, varying from an average of 36.3° C. a. m. to 36.9° C. p. m.

“Many of these patients when convalescent had an increase of half a degree, night and morning.

“Surgeon-Major Giles suspected that many of his patients in Assam had suffered from fever-at the onset of their malady, and he was confirmed in this impression by observing pyrexia in the monkeys he fed on anchylostoma embryos. After eliminating all those who had fever in the hospital, or a history of intermittent fever or any enlargement of the spleen, I found that 68 per cent of the remaining stated that their trouble had begun with a few days’ fever.” (Sandwith, 1894, p. 12.)

RESPIRATORY SYSTEM.

NOSTRILS.

See page 66.

RESPIRATION.

Many patients complain of a difficulty in breathing, especially after exertion. This symptom is quite natural, in view of the low condi-

tion of the blood and the emaciation of the muscles. Respiration is rather variable and does not appear to be a symptom of very great value; it may be slow, or it may be increased to about 30 or more per minute. According to Sandwith (1894, p. 12) dyspnea and noises in the ears were present, as might be expected with marked anemia.

MUSCULAR SYSTEM.

EMACIATION.

A progressive emaciation is more or less common, especially in severe cases. The arms and legs seem to be reduced to skin and bones; the chest is so emaciated that the ribs are very prominent and the beating of the heart is very evident. What little muscle is left is soft and flabby.

Emaciation is, however, not present in all cases, and even in some medium infections the muscles may be well formed and more or less hard. I recall one case in particular: A boy about 14 who showed a heavy infection microscopically, a clear clinical history of uncinariasis of several years standing, decided anemia, distinct cervical pulsations, abdomen rather distended ("pot-bellied"), yet his arms and legs were well formed and his muscles surprisingly solid for a patient in his condition.

Sandwith (1894, p. 13) found the average weight of 100 grown men upon admission to the hospital to be 117.5 pounds; the average height of these men was 5 feet 5.5 inches, which by Dawson's tables should scale at least 135 points. Of the patients who stayed in the hospital more than two weeks 70 per cent gained weight, 22 per cent lost, and 8 per cent remained stationary. The average loss of weight was 3.2 pounds, and was, of course caused by the necessary starvation, thymol, and purging. The average gain was 5.4 pounds, some patients gaining as much as 15, 17, 18, or 20 pounds.

GREAT PHYSICAL WEAKNESS.

One of the most pronounced symptoms complained of is a general weakness. The patient states that he is obliged to rest after exertion. In light cases a feeling of lassitude is experienced without being able to assign it to any particular cause; as a result, it is generally assigned by other people to laziness. In medium cases the patient may be able to work one to three or four hours before becoming exhausted; in very severe cases he will scarcely be able to walk across the room, or he may be confined to the bed for weeks at a time. A physical examination usually shows an emaciation proportionate to the weakness.

DIGESTIVE SYSTEM.

LIPS, GUMS, TEETH, AND TONGUE.

See p. 66.

NAUSEA.

Nausea was not noticed. Sandwith (1894, p. 11) states that vomiting and nausea are rarely complained of.

APPETITE.

The appetite may be light or ravenous. According to Sandwith, the appetite is invariably affected, sometimes ravenous at beginning, but later always capricious and diminished. The English nurses report to him that the patients were always begging for medical comforts or cigarettes, even in the middle of the night, when other patients were asleep. Among 40 men carefully examined, 16 said that their appetite was once greatly exaggerated, 16 pleaded diminution from the beginning of their illness, and 8 believed that their appetite was normal until the anemia became very marked.

Perverted appetite, "Dirt-eating."—The most important point to be noticed in connection with the appetite is the abnormal desire for some particular article of food. Frequently this is a preference for something sour or bitter.

Many patients with uncinariasis are known throughout the village or county as being especially fond of pickles. I have seen boys and girls in advanced cases of this disease who would greedily devour an entire bottle of pickles. Some patients are especially fond of sucking lemons, or lemons and salt, or salt alone. Others are known for their desire to chew coffee, or to drink large quantities of strong coffee without milk or sugar. Some are abnormally fond of buttermilk. Others are noted as "resin-chewers." Some are accused of "lapping sand." Many are accused of eating clay or dirt.

Dirt-eating has been discussed by a number of authors, opinion being divided as to its status. Some writers look upon it as the cause of the disease; others view in the habit only a symptom or a result; still others consider it nature's treatment of a diseased condition.

Among helminthologists there seems to be the impression that dirt-eating is especially likely to lead to infection with parasites. Among Southern physicians I found the idea quite prevalent that dirt-eating was one of the causes of the condition which I have classed as extreme uncinariasis.

During the trip now under discussion, I have had opportunity to observe many so-called dirt-eaters. As most authors state, it is exceptional that one will acknowledge that he eats dirt. I believe the explanation of this denial is very clear, namely, not only is there a certain amount of disgrace connected with the reputation of being a dirt-eater, but probably not over one person in ten, or possibly

in twenty, accused of eating dirt ever does so. The other nine to nineteen have their abnormal appetites developed in a different direction, namely, pickle-eating, lemon-sucking, coffee-chewing, resin-chewing, etc.

Among the articles eaten by these "dirt-eaters," various authors mention charcoal, chalk, dried mortar, mud, clay, sand, gravel, stones, shells, rotten wood, cloth, garments, paper, tobacco pipes, mice, young rats, etc.

It is, I believe, an error to attempt to reduce this abnormal habit to any one common basis. In general, however, it may be stated that the alleged "dirt-eating" in this country practically represents the severe cases of uncinariasis. To attempt to reduce dirt-eating to infection with worms, particularly with *Uncinaria americana*, will doubtless be thought extreme, more particularly by Northern physicians. Still the idea is not a new one, and a moment's consideration will show that this view is far less extreme than it at first appears.

For an excellent general discussion of dirt-eating, with extensive references to literature, the reader is referred to Le Conte (1845). For the purpose of the present paper it will suffice to call attention to certain facts and analogies. The habit of eating slate pencils, paper, and other objects by chlorotic girls is more or less commonly known. Pregnant women, also, may develop an abnormal appetite, which takes different phases, including dirt-eating. It is recorded that the Javanese women eat certain dirt in order to improve their appearance. In certain localities in tropical America (Orinoco) the natives eat earth during the overflow of the river when they can not obtain their regular food. Earth eating is said to be common and not injurious in certain parts of Africa. According to Sandwith (1894, p. 9), on the day of the maximum high Nile, and the general rejoicings thereupon, the town crier, who is on the lookout for backsheesh, presents "teen ibliz" (Nile mud) with a lemon to the inhabitants for luck, and many of them eat of it. Dogs, horses, cattle, hogs, and alligators are recorded as eating clay and sticks. The Alaskan seals, when infected with round worms, eat pebbles. Elephants, when infected with flukes, eat a certain kind of clay until a looseness of the bowels is produced. I have frequently heard Texas grangers attribute the death of cattle to eating sand, and in post-mortem examinations of cattle, sheep, and goats, in an anemic condition from intestinal worms (verminous gastritis caused by *Haemonchus contortus*, *H. Ostertagi*, etc., and infection of small intestine with *Uncinaria trigonocéphala* and *U. radiata*), I have repeatedly noticed in the stomach and intestine large quantities of sand; so that the farmers present declared that this was the cause of death. Dogs infected with intestinal worms eat grass. Cats also frequently eat grass, probably from the same cause. Children infected with eel worms (*Ascaris lumbricoides*) are known to

occasionally eat dirt, and I know of one such case where the habit ceased when the worms were expelled.

In view of the comparisons cited, it would seem that the idea of considering dirt-eating as a manner of infection with parasitic worms, although conceivable for some cases, is hardly correct as applied to most instances. That dirt-eating is an abnormal appetite due to a diseased condition (anemia and a disordered digestive system) as suggested by several authors as early as the first half of last century, seems to me to be an explanation of much more general application; and that this anemia and enteritis or gastritis may be produced by parasitic worms is an established fact. In this connection, it is interesting to note that Hancock (1831, p. 67), in discussing dirt-eating, mentions "worms preventing the nourishing effects of food;" Imray (1843, p. 310) remarks that "worms in considerable numbers were not uncommonly accumulated in the intestinal canal." Further, it is significant that various authors, in discussing the treatment of dirt-eating, attribute more or less success to certain drugs which are in fact used more or less in treating for intestinal parasites. Thus, Cotting (1836a) refers, as stated above, to the decrease of dropsy and of dirt-eating corresponding to the more general use of calomel; sulphate of iron is mentioned by Cragin (1836a), Pollard (1852), and others; according to Pollard (1852), copperas is a popular and successful remedy among the negroes; Hancock (1831) refers to a remedy containing arsenic as having had great success; Jordan (1832) states that dirt-eating decreased upon destroying the huts and moving the families to some other location.

To summarize: While it would seem decidedly extreme and unwarranted to maintain that dirt-eating is necessarily an indication of infection with intestinal worms, still I believe the conclusion is justified that it is undoubtedly a more or less common tendency in such infections, not only in man but also in other animals. It may be classed with the chewing of slate pencils, resin, coffee, sucking of lemons and salt, etc., as an abnormal appetite due to the anemia and abnormal condition of the intestinal tract. Further, for all practical purposes it is not much of an exaggeration to look upon most, if not all, so-called dirt-eaters of the sand areas of our Southern States as representing severe cases of uncinariasis.

Sandwith (1894) states that 26 per cent of his patients confessed to eating earth, and he refers to "earth hunger" as sometimes the cause and sometimes the effect of hookworm disease.

PAIN IN THE STOMACH; INDIGESTION.

Many patients complain of colicky pains "in the stomach," and will indicate the region between the navel and the ensiform cartilage as the seat of the "misery." Indigestion is frequently mentioned, and the tongue is occasionally coated.

Just how much the indigestion is due to uncinariasis and how much to other causes may be considered an open question. Foul breath is mentioned by some authors as a common symptom of uncinariasis, but this has not been particularly noticeable in many of the cases I saw.

Many authors explain the tendency to dirt-eating as an effort to neutralize the hyperacidity of the stomach. As I have just shown (p. 74), however, many patients with uncinariasis eat pickles and suck lemons.

According to Sandwith, a gnawing, throbbing pain in the epigastrium is the first symptom complained of, chiefly because it is constant, whereas a severe colic and borborygmi (rumbling of bowels caused by gas) of intestine are present from time to time. I was unable to confirm the constancy of the pain.

CONSTIPATION AND DIARRHEA.

Sandwith (1894, p. 11) states that when the patient is not under thymol and purgative treatment, constipation is a very constant symptom in hospital cases: 60 per cent had suffered for a long time from obstinate constipation, 28 per cent had had diarrhea before admission, and 12 per cent had no recollection of being troubled with either. "None of the figures depending upon the memory of the patients must be taken as absolute truth, as the intelligence of many is of a very low order." Diarrhea, and even dysentery, are not uncommon in very advanced cases, especially those complicated with *Bilharzia* (= *Schistosoma*, which has not been reported as endemic in the United States), or ulceration in the rectum; and unless the patient is robust enough to support thymol, such cases are apt to end fatally.

In my own cases I found both constipation and diarrhea, but I am not in a position to state that either symptom was regular or characteristic for any given degree or stage of infection. In severe cases diarrhea was certainly more or less common.

Feces.—See page 68.

NERVOUS SYSTEM.

The nervous symptoms usually develop later than either the intestinal or the circulatory symptoms.

EYES.

See page 65.

EARS.

According to Sandwith (1894, p. 12), noises in the ear are present. None of my patients complained of this symptom.

MENTAL LASSITUDE, HEADACHE, DIZZINESS, AND NERVOUSNESS.

Not only does physical exertion result in exhaustion, but mental exertion has to be avoided. The children complain that they are unable to study and that any continued application to books results in

severe headache. This feature of the disease is fully confirmed by the testimony of both teachers and parents, who assert that children of this class are usually much more backward (and even stupid in their studies) than other children not showing the symptoms under discussion.

Dizziness is very commonly mentioned by the patients. This feeling, which they usually speak of as a "swimming in the head," is experienced especially upon rising suddenly from a chair or a bed.

Nervousness does not seem to be so commonly complained of as mental lassitude, headache, and dizziness. Still it is more or less frequently mentioned, more particularly by the girls and women.

Among girls from about 13 to 20 years of age it was quite noticeable that they were more timid and more emotional than were their healthier sisters.

According to Sandwith (1894, pp. 11-12), there is pain in the head, generally referred to the temples, while in the knees there is almost invariably present great weakness and some pain; occasionally there is in addition pain in the shoulders. Giddiness is another very general symptom, and it is this as much as anything else which compels the patients to give up work. The worst cases are those which are nearly always asleep, and can not be interested in anything when they are awake. On the whole, sleepiness is decidedly a symptom. Dense stupidity, associated sometimes with reiterated demands for a favor already granted, shows that the bloodless brain is affected in all advanced cases, and at least three times Sandwith (1894, p. 13) found a condition of weak-mindedness which would have warranted the patients being sent to the asylum.

PATELLAR REFLEX.

Absence of patellar reflex is reported in cases of general debility and muscular weakness. Sandwith (1894, p. 13) found this reflex unaltered in 35 per cent of the cases examined, completely absent in 48 per cent, decidedly diminished in 5 per cent, and a little exaggerated in 12 per cent, all of which were early cases.

GENITAL SYSTEM.

EXTERNAL GENITALIA; SEXUAL FUNCTION; MENSTRUATION; STERILITY.

In cases where infection has taken place in early childhood, the delayed development of the genital system is very marked. Patients of 16 to 22 years of age may not be better developed than healthy persons of 11 to 15 years. Menstruation may be very irregular, especially in summer. This same condition is insisted upon in many early writings on dirt-eating. Mothers frequently ascribe the condition of their daughters to the absence or irregularity of the menstruation as is mentioned also in early writings on dirt-eating.

Sandwith (1894, p. 13) found impotence to be a decided symptom in hookworm disease. Of 38 men especially examined on this point, 24 had completely and 5 had almost entirely lost their virile power, while of the remaining 9 men, 5 aged from 19 to 25 had their puberty considerably delayed.

TENDENCY TO ABORTION.

Among women affected with uncinariasis I found a marked tendency to abortion. Given a woman about 28 years old who had been married nine years—a not uncommon history is that she has had 3 to 5 children and 3 to 4 miscarriages, and she looks to be about 50 years old. Not being able to follow these cases through their entire medical history and the history of their husbands, I must leave the question open as to how many of these abortions are to be attributed to uncinariasis and how many are due to other causes. In the country districts I was thrown in with the anemic not with the healthy families, hence I have no good basis for comparison of these two classes for the particular localities visited. In some cases a history of venereal disease was suspected or admitted; in others, the abortion came on after pitching fodder; in some cases the patients had taken more or less quinine during their life, under the supposition that they had malaria; and in still other cases, my suspicions were aroused in other directions. The determination of the exact relation of uncinariasis to the miscarriages, which are certainly strikingly prevalent, must be left to those who can follow the cases for a longer period of time.

PREVALENCE OF UNCINARIASIS IN THE UNITED STATES.

In several earlier papers (1901, p. 524; 1902a, p. 778; 1902b, pp. 183, 212) I have advanced the view that uncinariasis must be more common in this country than is generally supposed. In my preliminary report on this trip (see above, p. 35), I said that: "There is in fact not the slightest room for doubt that uncinariasis is one of the most important and most common diseases of this part [South Carolina] of the South, especially on farms and plantations in sandy districts."

Harris (see above, p. 36) went even farther than this and claimed that uncinariasis is "the most common of the severe diseases of the South."

In considering the subject of the frequency and economic importance of the disease under discussion, I do not wish to seem to underestimate the prevalence of tuberculosis and of venereal diseases among the negroes or of malaria among the whites. Further, I recognize the fact that at the present moment an exact mathematical estimate can not be made. Speaking in general terms, however, the facts at my disposal at present seem to indicate that taking the Southern Atlantic

States as a whole, uncinariasis must be considered as one of the most common and widespread maladies; in frequency it belongs in the general class with malaria, tuberculosis, and gonorrhea.

In cities and in rural clay districts it is probably less common than any one of these three maladies, for such localities may present local foci of infection for the diseases in question, while the local foci of infection with uncinariasis are much more limited.

Among the negroes of the rural sand districts, uncinariasis seems to be much less common than either tuberculosis or gonorrhea. Its apparent rarity may, however, be deceptive (see p. 51).

Among the whites of the rural sand districts, uncinariasis is apparently the most common disease found. Nevertheless, in some sand districts, probably with a clay or other impervious subsoil favorable to the formation of marshes, malaria rivals uncinariasis for first place.

From these qualified statements it will be seen that I do not feel justified in adopting the view advanced by Harris, namely, that uncinariasis is "the most common of the severe diseases of the South."

In all probability, further study will show that in Mexico, Central America, and parts of South America, hookworm disease is more important and more common than in the United States.

Sandwith (1894, pp. 5-6), in discussing the frequency of this disease in Egypt, says:

"It is impossible to know what amount of the population [of Egypt] is affected, but the statistics of the recruiting commissioners for 1892 are worth quoting. Nearly every adult male peasant is liable for conscription, and the conscripts are immediately examined in their villages. In upper Egypt 5,988 men were called, and 200, or 3.3 per cent, were rejected for anæmia. In lower Egypt 661, or 6.2 per cent were rejected from this cause out of 7,420 men. Every province furnished anæmic rejections, but Menoufieh came highest on the list with 13.9 per cent, while I find from hospital statistics that no less than 15 villages in that province are infected. The recruiting medical officer, who is an Englishman, only rejects those who are obviously too anæmic to serve with the colors, accepting many who are already the hosts of the bloodsucking worm. Thus the medical reports for the Egyptian army show that in 1890 there were 114 admissions to the hospital for anæmia, in 1891, 107 admissions, and in 1892, 170 admissions. In 1891, 22 soldiers were invalided from the service for anæmia, and 65 in 1892, besides 1 death. The number of admissions for debility is equal to those for anæmia, and doubtless includes many cases of anchylostomiasis."

Dobson (1893, p. 63), examined 547 of the healthiest looking coolies from India and found hookworms in no less than 454 of them.

CLINICAL DIAGNOSIS OF HOOKWORM DISEASE.

As stated above, a man who is familiar with this disease should have no difficulty in recognizing severe cases, especially if he is in the area of infection. In light and medium cases, however, it is unsafe to make a diagnosis upon symptoms alone, unless such cases are associated

in the same family or neighborhood with severe cases. The best and most reliable method of diagnosis is by fecal examination, although in blood examination increased eosinophilia indicates the possibility of intestinal parasites.

First of all let us recall that uncinariasis is a possibility which should be considered in connection with all cases of anemia, especially among earthworkers, as in miners, brickmakers, canal diggers, farmers, etc., or in persons returning from the tropics, and among persons who have a history of residence on sandy soil. Three methods of fecal examination are open to us—the blotting-paper test and the microscopic and the gross examinations.

BLOTTING PAPER TEST WITH FECES.

For persons who are not in a position to make a microscopic examination, the blotting-paper test (referred to on p. 69), will be found very useful. To make the test, use only fresh feces. Place an ounce or more of the stool on a piece of white blotting paper (any absorbent white paper will answer the purpose); allow it to stand for twenty to sixty minutes; remove the feces and examine the color of the stain. In about four out of five cases of medium or severe uncinariasis, the stain is reddish brown and immediately reminds one of a blood stain. In making this test on anemic patients, piles should of course be excluded.

It developed in my work in the Virginia penitentiary, that this test is open to error in dealing with criminals. In order to avoid work, convicts, especially hard-labor contract convicts, occasionally produce a hemorrhage purposely by wounding the mucosa of the rectum by means of some sharp instrument.

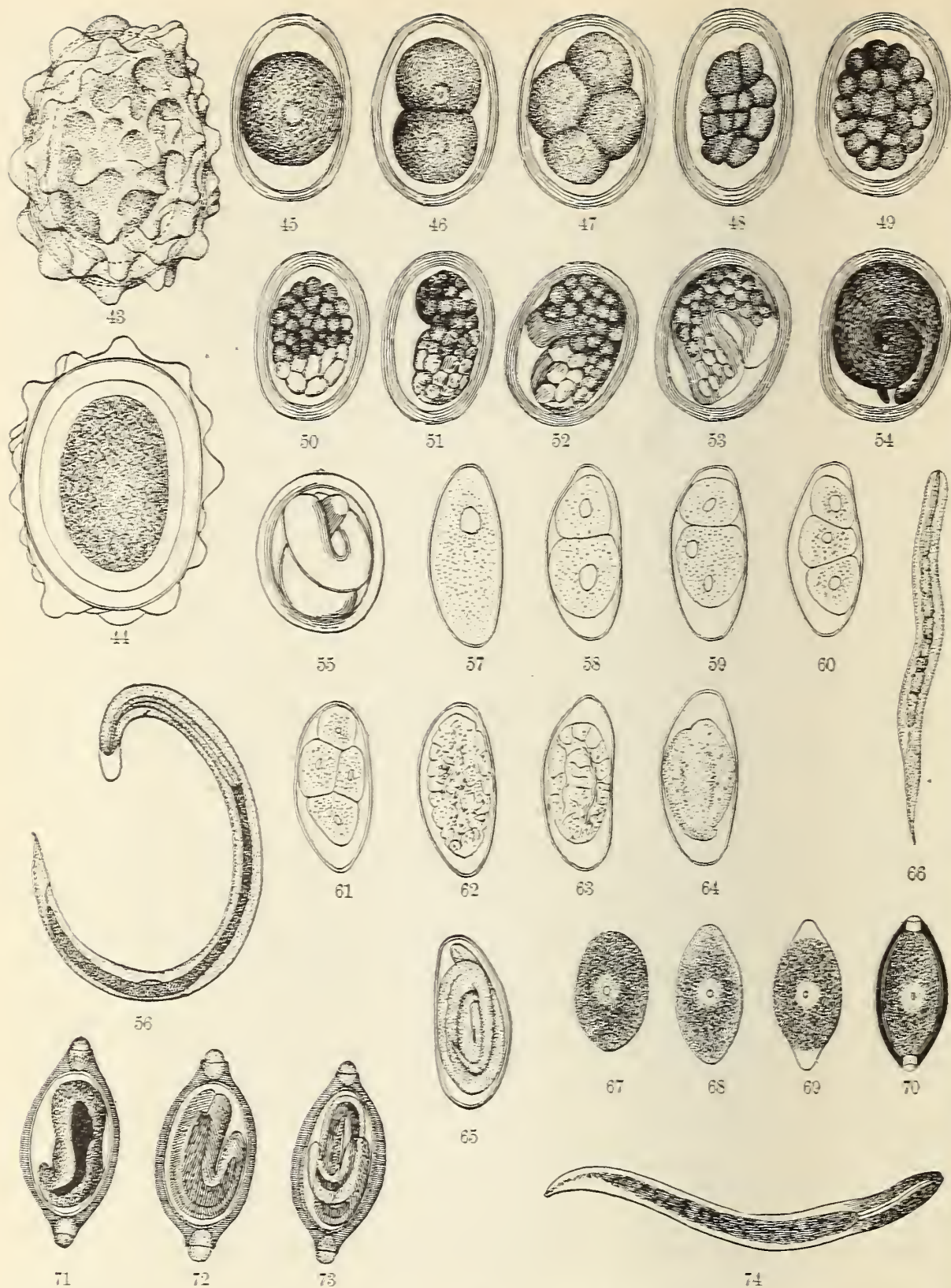


FIG. 43.—Egg of the common ascaris (*Ascaris lumbricoides*) of man, as found in feces. Seen with superficial focus. Greatly enlarged. (After Stiles, 1902b, p. 202, fig. 158.)

FIG. 44.—The same, as seen with median focus. Greatly enlarged. (After Stiles, 1902b, p. 202, fig. 159.)

FIGS. 45-54.—Embryology of the common ascaris (*Ascaris lumbricoides*) of man, showing the changes undergone by the egg after being discharged in the feces. (After Leuckart, 1867, p. 213, fig. 154.)

FIG. 55.—Embryo of the common ascaris (*Ascaris lumbricoides*) of man, in the eggshell. (After Leuckart, 1867, p. 215, fig. 156.)

FIG. 56.—Free embryo of the common ascaris (*Ascaris lumbricoides*) of man, casting its skin. (After Leuckart, 1867, p. 214, fig. 155.)

FIGS. 57-64.—Embryology of the common pinworm (*Oxyuris vermicularis*) of man, showing the changes undergone by the egg while in the female worm. (After Leuckart, 1868, p. 322, fig. 191.)

FIG. 65.—Embryo of the common pinworm (*Oxyuris vermicularis*) of man, in the eggshell, as found in fresh feces. (After Leuckart, 1868, p. 328, fig. 196.)

FIG. 66.—Full-grown embryo of the common pinworm (*Oxyuris vermicularis*) of man, after it has escaped from the eggshell. (After Leuckart, 1868, p. 328, fig. 195.)

FIGS. 67-70.—Egg of the common whipworm (*Trichuris trichiura*) of man, showing changes undergone while still in the female worm: fig. 70 is the stage found in fresh feces. (After Leuckart, 1868, p. 491, fig. 275.)

FIGS. 71-73.—Later stages of development of an allied whipworm (*Trichuris affinis*) of sheep and cattle, showing changes after the egg escapes in the feces. (After Leuckart, 1868, p. 494, fig. 276.)

FIG. 74.—Isolated embryo of *Trichuris affinis*. (After Leuckart, 1868, p. 495, fig. 277.)



FIG. 75.—Egg of Cochin-China diarrhea worm (*Strongyloides stercoralis*) found in stools. (After Thayer, 1901, pl. 9, fig. A.)

FIG. 76.—Rhabditiform embryo of same, from the stools. (After Thayer, 1901, pl. 9, fig. B.)

FIG. 77.—Filariform larva of same derived, by direct transformation, from a rhabditiform embryo. (After Thayer, 1901, pl. 9, fig. C.)

Figures 75 to 77 were drawn from life, as seen under Leitz, objective 7—ocular 3.

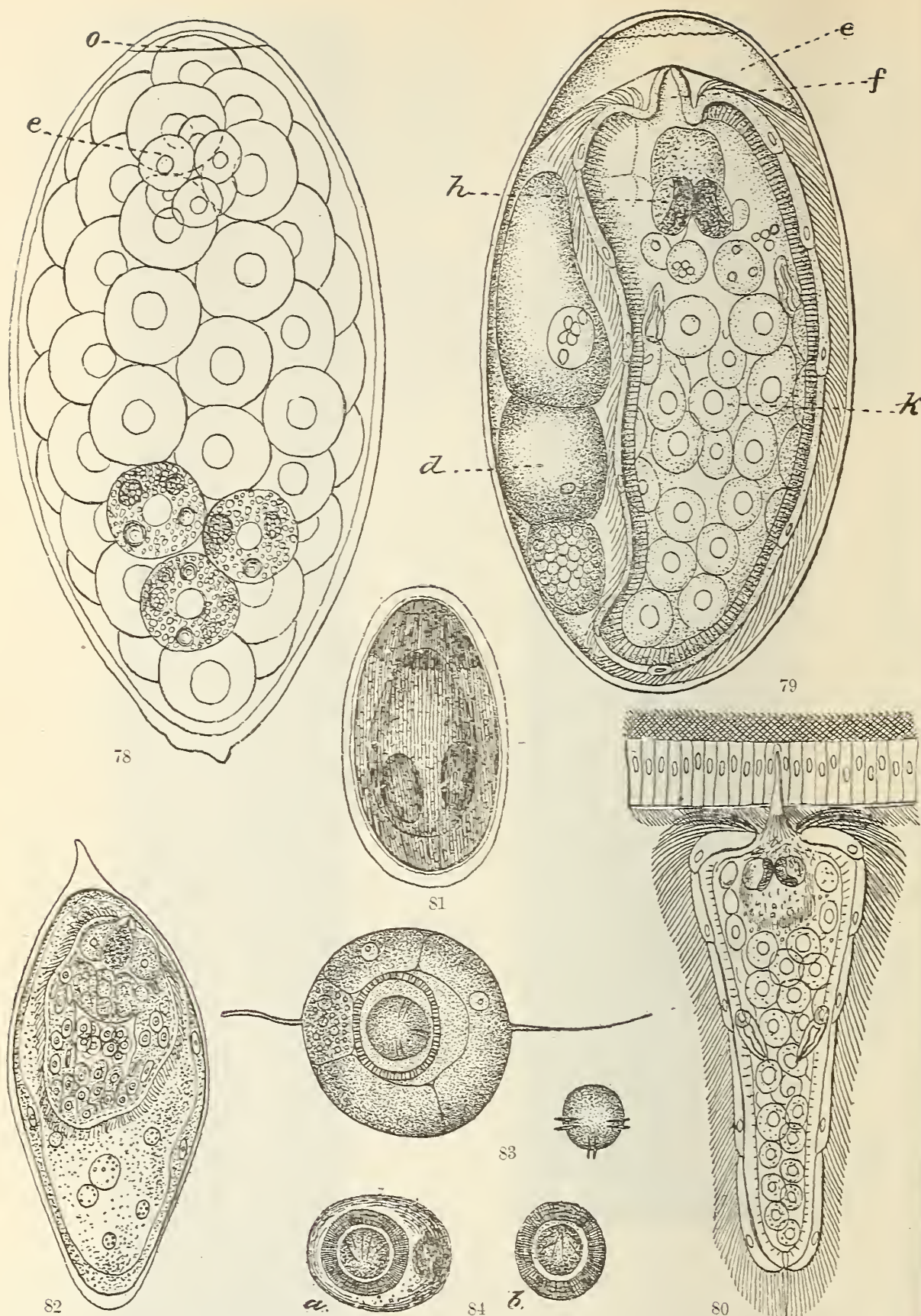


FIG. 78.—Egg of the common liver fluke (*Fasciola hepatica*) examined shortly after it was taken from the liver of a sheep; this is the same stage that is found in human feces: at one end is seen the lid or operculum, *o*; near it is the segmenting ovum; the rest of the space is occupied by yolk cells which serve as food: all are granular, but only three are thus drawn. X 680. (After Thomas, 1883, p. 281, fig. 1.)

FIG. 79.—Egg of the common liver fluke containing a ciliated embryo (miracidium) ready to hatch out; *d*, remains of food; *e*, cushion of jelly-like substance; *f*, boring papilla; *h*, eye-spots; *k*, germinal cells. X 680. (After Thomas, 1883, p. 283, fig. 2.)

FIG. 80.—Embryo of the common liver fluke (*Fasciola hepatica*) boring into a snail. X 370. (After Thomas, 1883, p. 285, fig. 4.)

FIG. 81.—Egg of lancet fluke (*Dicrocoelium lanceolatum*) with contained embryo. X 700. (After Leuckart, 1889, p. 379, fig. 171.)

FIG. 82.—Egg of human blood fluke (*Schistosoma haematobium*) with contained embryo, passed in the urine or in the feces. X 285. (After Looss, 1896, pl. 11, fig. 112.)

FIG. 83.—Egg of beef-measle tapeworm (*Taenia saginata*) with thick eggshell (embryophore), containing the six-hooked embryo (oncosphere) enlarged. (After Leuckart.)

FIG. 84.—Eggs of pork-measle tapeworm (*Taenia solium*): *a*, with primitive vitelline membrane; *b*, without primitive vitelline membrane, but with striated embryophore. X 450. (After Leuckart, 1880, p. 667, fig. 297.)

No special technique is necessary. Simply take a small amount of feces, preferably from near the surface, about the size of the head of a large pin; spread this out in a drop of water on an ordinary microscopic slide and cover the preparation with a cover slip. Examine under any moderately high power, as a Zeiss 8 mm., Zeiss C, or a Bausch & Lomb one-third inch. Look carefully, with not too strong illumination, for an elongate oval egg with thin shell, and with protoplasm either unsegmented or in the early stages of segmentation. The older the feces and the warmer the weather the more advanced will be the segmentation. In case of infection with *Uncinaria americana* the fully developed embryo may be found within the eggshell. Be cautious not to mistake for the egg of the *Uncinaria* the eggs of *Ascaris lumbricoides*, which have a thick gelatinous, often mammillated, covering and an unsegmented protoplasm (figs. 43-44), or the eggs (figs. 57-65, of *Oryzias vermicularis*, with a thin asymmetrical shell (one side being almost straight) and containing an embryo, or the eggs of whipworms (*Trichuris trichiura*, more commonly known to physicians as *Trichocephalus dispar*), possessing a smooth, thick shell, apparently perforated at each pole, and an unsegmented protoplasm (fig. 70).



FIG. 85.—Egg of the dwarf tapeworm *Hymenolepis nana* of man. Greatly magnified. (After B. H. Ransom, unpublished.)

As a rule, in fecal examination I prefer to use the thick, large, 2 by 3 inch slide, such as is used in examining for trichinae, rather than the ordinary 1 by 3 thin "English slide." The larger slide is not only more steadily and more easily manipulated in case one is working without a mechanical stage, but it is much cleaner to handle.

In most cases of infection with intestinal worms the simple method just described will suffice for a positive diagnosis. Before giving a negative opinion, however, I invariably make ten preparations or follow a procedure which we may call "sedimenting the feces." Experience has shown me that in cases of negative diagnosis by the simple method positive diagnosis occasionally results if the feces are washed and "sedimented."

Method of washing and sedimenting feces.—Take one or two ounces of feces, fresh or dry, mix with water, and place in a large bottle, retort, jar, or any other receptacle; add enough water to make from a pint to two quarts, according to the amount of feces; shake or stir thoroughly and allow to settle; pour off the floating matter and the water down to near the sediment; repeat the washing and settling several times, or as long as any matter will float. The last time this is done use a bottle or graduate with a smaller diameter, and when the material is thoroughly settled examine the fine sediment. It will be

found that the eggs have settled more numerous in the fine sediment than in the coarse material.

In case an unusual amount of large coarse material is present in the feces, it is sometimes convenient to pour the entire mass through a sieve, rejecting the portion left in the sieve; or to wash the feces in a sieve, holding the latter under water. As a rule, however, the sieve is not very useful in fecal examinations.

The centrifuge does not appear to be of any special value in fecal examinations.

If facilities are not at hand for making a microscopic examination, about half an ounce of either perfectly fresh feces, or of rather dry feces, may be placed in a bottle, preferably with a large neck, properly packed in a mailing case, and sent to any professional pathologist or zoologist for examination.

GROSS EXAMINATION OF FECES.

If uncinariasis is suspected and it is not practicable either to make a microscopic examination or to delay matters until a specimen can be sent away for examination, still another method of diagnosis is possible. Give a small dose of thymol, followed by salts, and collect all of the stools passed. Wash the stools thoroughly several times in a bucket, and examine the sediment for worms about half an inch long, about as thick as a hairpin or hatpin, and with one end curved back to form a hook. If these are found, institute definite treatment.

TREATMENT OF HOOKWORM DISEASE.

ANTHELMINTHIC TREATMENT.

The two drugs most commonly used in uncinariasis are thymol and male fern. The day before treatment the patient is placed on a milk and soup diet for three days.

Thymol.—The directions usually given for thymol treatment are these: Two grams (31 grains) of thymol at 8 a. m.; 2 grams (31 grains) at 10 a. m.; castor oil or magnesia at 12 noon.

One week later the stools should be examined, and if eggs are still present, treatment should be repeated until the eggs disappear, but it is not best to give the thymol more than one day per week. Some cases of hookworm disease are quite obstinate and require a treatment extending over several weeks. It is, therefore, an unfortunate error to expel a few worms with one or two doses and then dismiss the patient as cured without having made further microscopic examination for eggs.

Sandwith (1894, p. 21) reports 42 men cured after a single dose; 58 after 2 doses; 43 after 3 doses; 25 after 4 doses; 9 after 5 doses; 4 after 6 doses; 2 after 7 doses, and 2 after 8 doses.

A number of writers, particularly Giles, Sandwith, and others, agree that small doses of thymol are valueless, but Sandwith is of the opinion that 4 grams in 24 hours are as efficacious as 6 grams, and the former dose is certainly less dangerous.

Worms may be found in the stools as early as eight hours after the first dose of thymol. In 50 cases Sandwith counted 1,301 worms in stools passed within eight hours after the first dose and 444 worms within the next sixteen hours. From his context, however, it is clear that brandy was given to these patients (see below), hence the thymol was dissolved more rapidly. Giles reports that he seldom found any worms until twelve hours after the first dose of thymol. "Occasionally patients vomited after swallowing thymol, but as a rule they retained it perfectly," and "they used to ask for an extra dose of it while convalescing."

Sandwith goes on to say that "large doses of thymol have a poisonous effect on the system, not unlike those produced by carbolic acid. The temperature is lowered one or even two degrees centigrade, and both pulse and respiration are slowed. The patient remains for a few hours collapsed, giddy, and faint, and has to be kept lying down, but at sunset he is quite well again and asking for food." He gives the following as a typical case:

"*January 14, 1892.*—6 a. m., temperature 37.5°, pulse 80, respiration 19; patient in his usual state and was given 2 grams of thymol. 7 a. m., temperature 37°, pulse 80, respiration 19; says he has slight nausea, giddiness, and colicky pains in the epigastrium. 8 a. m., 2 grams more of thymol given. 9 a. m., temperature 35.5°, pulse 70, respiration 17; great giddiness, can not stand or walk; very sleepy, and talks like a drunken or very sleepy man. 12 a. m., symptoms much the same; sweating while asleep. 2 p. m., temperature 37.5°, pulse 75, respiration 18; apparently quite well again. Says he does not mind the thymol, except that it makes him lose consciousness."

Some authors advise the use of alcohol with thymol, others warn against such use. Sandwith, in referring to this subject, says: "Warned by the death of at least one of my patients immediately after digesting the thymol, I have always administered to feeble men 25 grams of brandy with each 2 grams of thymol, with the happiest results."

Authors who warn against the use of alcoholics during treatment do so on the ground that thymol is soluble part for part in alcohol, hence will more quickly be absorbed by the system. With one of my assistants (see Stiles & Pfender, 1902a), in the Bureau of Animal Industry, I treated a number of dogs with thymol in various forms. We gave doses varying from 10 to 100 grains (0.648 to 6.48 grams) to dogs weighing from 8 to 35 pounds. In alcoholic solution, 30 grains (1.94 grams) caused convulsions in a dog weighing 15 pounds, and severe convulsions in one weighing 14 pounds; on the other hand, an 8-pound, old dog suffered no ill effects after 30 grains in alcohol, and a 35-pound

dog took 75 grains (4.86 grams) in alcohol without deleterious effect. In tablet form, without oil, a single dose of 100 grains (6.48 grams) killed a dog of 15 pounds in four days; a dose of 50 grains caused a 12-pound dog to attempt to vomit; a dose of 75 grains was without appreciable effect in a dog of 35 pounds; 30 grains were without effect on a dog of 8 pounds; daily doses of 20 grains each caused a slight diarrhea after six days in a dog of 15 pounds; the same dose caused a 17-pound dog to be quite sick on the sixteenth day. In tablet form, with castor oil, 30 grains caused a 12-pound dog to attempt to vomit, while doses of 10 and 20 grains were negative on dogs weighing 12 to 15 pounds. In powdered form, without oil, 20 grains caused a 12-pound dog to attempt to vomit, while doses of 10 and 20 grains respectively were negative in dogs of 12 and 15 pounds; 40 grains caused no ill effects in a dog of 8 pounds, and a dose of 80 grains was without effect on a dog of 35 pounds. In powdered form, with castor oil, 20 grains caused slight convulsions in a dog weighing 15 pounds, but doses of 10 and 20 grains were negative on dogs of the same weight. Blue foxes to which we gave 3 grains in alcohol became quite sick. Theobald states that a dose of 3 grains has produced partial prostration in a bull-terrier, but he does not give the weight of the dog.

According to Sandwith, the contra-indications for thymol are "excessive debility, very low temperature, age above 60, and advanced diseases of the heart or any other organ. Boys take it very well in half quantities."

Sandwith states (1894, p. 17), that of 8 fatal cases treated with thymol 2 died, he thinks, in consequence of the thymol, eleven and forty-eight hours, respectively, after taking the dose; both of these men had previously had thymol without bad effect, but they were both in a miserable state of exhaustion and debility. He does not think that thymol accelerated the deaths of any of the remaining 6 cases, which occurred five, six, nine, thirteen, fifteen, and nineteen days, respectively, after the last dose of thymol. Three of these cases were over 65 years of age.

While my experience with thymol in man is very limited, I must confess that from my experiments upon animals, I am afraid of the use of alcoholics *per os* during treatment, and in the case of weak patients I should be more inclined to use a stimulant hypodermically than run the risk of dissolving the thymol too rapidly or in too great quantity at one time. Furthermore, it is at least doubtful whether our American hookworm will be so difficult to expel as is the Old World species, because of the absence of the ventral hooks (cf. figs. 5 and 10) in *Uncinaria americana*.

In the case of "excessive debility" and other conditions which Sandwith designates as contra-indications for thymol, it is not clear what drug Sandwith would use, for he states (1894, p. 20) that "for

the last three years I have looked upon it as a waste of time to administer any other anthelmintic than thymol for this parasite" [*Ancylostoma duodenale*]. Certainly persons in the conditions described should not be allowed to go untreated, and despite the view advanced by some authors, smaller doses of thymol, repeated one day per week and extending over several weeks, may be expected to yield some results.

Male fern.—Several European authors advise the use of large doses of extract of male fern in treating hookworm disease. Eichhorst's (1901, p. 314) recent "Practice" places the dose at 10 grams (2.5 fluid drams) to 20 grams (5 fluid drams). It has been pointed out by Lépine (1891a, 1891b) and others that such large doses of this drug are likely to be followed by serious toxic symptoms and even by death. Lépine summarizes the conditions as (1) symptoms of gastrointestinal irritation characterized by the redness and the hemorrhages; (2) nervous symptoms (convulsions and paralysis); (3) albuminuria, and (4) glycosuria, and he warns not to give over 8 grams of the extract as a maximum dose. Hare gives the dosage of the extract as 4 to 8 grams (about 1 to 2 fluid drams). Male fern should be followed in three to four hours by a calomel purge, aided by a saline, but not by castor or other oils, as the latter increase the danger of absorption, hence of poisoning.

Calomel.—While thymol is at present considered the most reliable remedy in hookworm disease, indications are not lacking (see p. 76) that considerable good may be accomplished in the American form of the disease by the use of calomel. This drug will not, however, be followed by such prompt and satisfactory results as will thymol.

GENERAL TREATMENT.

The administration of thymol has for its object the expulsion of the parasite, hence the removal of the cause of the disease. This should be supplemented by efforts to build up the depleted system by means of good nourishing food, iron, etc. It is well to give the iron daily, except on the days that thymol is taken. Sandwith (1894, p. 25) claims that the blood was most benefited by a daily supply of 1.5 grams (23 grains) of the sulphate of iron in water in three equal doses.

PROGNOSIS.

Among physicians I found the view rather prevalent that the prognosis was poor for children who presented severe cases of the disease. This view is probably due to the fact that the cause of the trouble was not understood, hence treatment was not directed to removing the cause.

The proposition now before us is, first, to remove the intestinal parasites, and second, to build up the patients.

To accomplish the first desideratum may require some patience, but efforts will eventually be successful. The second point may also be carried out, unless, of course, the patient is too far gone at the time of treatment to recover from the effects of the disease.

In not all cases can it be expected that a dwarfed, emaciated, and stupid child can be immediately placed upon the same physical and mental basis as his brothers, but even such cases can be greatly improved.

LETHALITY OF HOOKWORM DISEASE.

I know of no extensive and exact statistics regarding the lethality of uncinariasis, and traveling as rapidly as I did, it was impossible to establish any definite facts from personal observation, since it was the exception that I saw any case more than once. Furthermore, owing to the fact that many light cases will escape attention, any lethality percentages published will probably be above the actual figures.

I doubt whether sufficient data are at hand to justify even approximate statements regarding the lethality of hookworm disease. That numerous cases, not properly treated, terminate fatally can not be doubted. Still, it is remarkable how low a person may be with hookworm disease and still live.

Among physicians I met with the most contradictory ideas on this subject. Several excellent observers maintained that all severe cases which reached an edematous condition were invariably fatal; equally keen observers doubted whether this disease was frequently the actual cause of death; the view was quite general that patients suffering from medium or severe attacks of this malady very seldom lived through even medium or light attacks of such diseases as typhoid fever, or pneumonia, and that severe attacks of malaria were frequently fatal; also that they were very uncertain patients in confinement.

In my own observations, several points seemed quite significant. In the first place, the large number of cases of long standing found in so many families did not indicate a high lethality. Further, several adults were seen who had formerly unquestionably presented severe infections, but who are now in a fairly good state of health. In one family with 9 children living, most of whom clearly presented light, medium, or severe infections, there was a history of death of 9 other children, but satisfactory answers as to the cause of these deaths were not obtained. That some of the children had died of uncinariasis is very probable. On the other hand, families were seen with 8 to 10 children, all or nearly all in an anemic condition, some with clear medium to severe cases of uncinariasis, yet without history of any fatal case in the family. I have heard of localities in Central America (but have not investigated them personally) where it is said that a dis-

ease, which from its description I believe to be uncinariasis, probably due to *Uncinaria americana*, causes an immense mortality among the children.

Taken all in all, the data obtained did not convince me that uncinariasis, *per se*, is so fatal a disease in man as is generally supposed. On the other hand, I obtained the impression that while very severe cases are not infrequently fatal, the general effects of the malady upon the system are of greater and more far-reaching importance than the lethality of the infection itself. In other words, if uncinariasis were eliminated, the lethality of other diseases, such as pneumonia, typhoid fever, malaria, and also of child birth, would be decreased, and in the sand and mixed sand and clay areas this decrease would not be an insignificant factor.

One physician stated to me that he was confident that he had lost several hundred patients from uncinariasis within the past forty years.

Sandwith (1894, pp. 16-17) states that of the patients nominally under his care, 89.5 per cent were cured or greatly improved, 2.5 per cent were unrelieved, and 8 per cent died.

"Most of the fatal cases had loud anemic murmurs, marked subnormal temperature, slight general edema, albuminuria, and great mental weakness.

"The actual cause of death was exhaustion, from utter absence of rallying power. It is difficult to believe that the pathological effects are induced only by hemorrhage from the daily suction of scores or even hundreds of worms. In addition to the loss of blood, we have general thickening and degeneration of the duodenum and jejunum, and consequent interference with normal digestion; then nonassimilation, and eventually a process of slow starvation. It is also worthy of consideration that there may be in prolonged cases some self-poisoning from the great number of bites in the walls of the intestines containing ill-digested and perhaps decomposing food."

POST-MORTEM APPEARANCES.

I did not have occasion to make any autopsies during the trip; hence I am unable to present any original observations in this line. For careful accounts of single cases of autopsies, the reader is referred in American literature to Strong (1901), Yates (1901), Claytor (1902a), and Capps (1903a).

Sandwith's (1894, pp. 17-20) summary of 26 autopsies is not accessible to many American physicians; hence it is quoted here in full.

"Some of the earlier autopsies were made by myself, the later ones by Dr. Kaufmann. The muscles were in one case described as of normal color, but in all others they were very pale. There was usually a great absence of subcutaneous fat. The lungs were very pale and edematous in all cases, and all the organs were extremely bloodless.

"In one case there was noted edema of glottis. The heart was found to be hypertrophied ten times [in 10 cases], and was very small twice, generally pale brown in color, and on three occasions there were marked changes in the mitral valve. The most common abnormality in the liver was a brownish-yellow fatty appearance.

In one case there were several abscesses and the liver weighed 3,700 grams, and in one patient there was well-marked jaundice.

"The spleen was enlarged in one-third of the post-mortems. The kidneys invariably showed some change, though this was often much more marked in one kidney than in the other. They were very pale in 24 patients, 3 of whom had several small cysts. Of the remaining 2 patients one had granular kidneys and the other had cysts, but the kidneys were of normal color.

"The brain was always exceptionally white, and in one case there was recent apoplexy.

"The small intestines showed, of course, the most important changes. As a rule, there were many hemorrhages and bites in the jejunum and ileum, but in one of the cases where the bites were carefully counted there were only 6 in the jejunum and ileum, and no worms were found. In another case, however, there were 575 bites in the small intestine, besides 250 anchylostoma. In yet another there were 100 bites, the farthest of which was 4.5 meters from the pylorus. In only two cases was there much liquid blood in the intestine. •

"I have not observed the constant changes in the mucous membrane of the stomach described by Giles. The great variability in the number of anchylostoma found at the autopsies is interesting. In 7 cases, all treated by thymol during life, no worms could be found. In an eighth case, also treated by thymol, but insufficiently, there were 10 worms. The remaining 18 cases had not had the advantage of thymol. Six of them, nevertheless, were found to have less than 10 worms, and in two of these corpses only 1 worm was found in each jejunum. Three other cases numbered 20, 40, and 50 worms, but the remaining 9 had numbers varying from 170 to 381, terminating with the maximum record of 863. On that occasion the autopsy was made seven hours after death, and the worms were scattered from a point 1 inch beyond the pylorus for the length of 3 meters; 217 of the 863 were attached still to the intestine and were surrounded by much bloody mucus, while 646 were lying free in the intestine; 16 of the latter were still alive, and one couple were in copulation.

"Next the position of the worms deserves notice. It was quite the exception to find any parasites in the duodenum. Can it be that when the duodenum becomes thickened and riddled, as it were, with the ravages of former generations, the anchylostomum fastens by preference on to the jejunum? Some such cause as this suggestion of gradually shifting the pasture is required to explain the interesting fact that it is not the most advanced cases of anemia which will always yield the largest quantity of worms. In such cases the parasite must not only have to burrow extra deep, but the blood when reached is, of course, deficient in quantity. Or is it that the half-starved worms are dislodged by repeated attacks of diarrhea? The furthest feeding ground that I have seen was 6.30 meters from the pylorus, where there was a worm firmly attached.^a But, as a rule, the attached worms are all within 2 meters of the pylorus, and have their heads and sometimes half their bodies buried in the mucous membrane. It is often impossible to dislodge them by a strong stream of water, and they must then be pulled out by forceps.

"I examined about 50 cases to see the proportion of male to female worms, and found it 56 to 44 per cent. This is not in accordance with some of the authorities, who say that males are always more rare than females, and that males are less influenced than females by the action of expellent drugs."

^a"Dubini has only once seen the worm in the ileum. The nearest feeding ground seen by me was 24 centimeters from the pylorus. In that case there were 100 worms attached and 281 detached, besides oxyurides."

PREVENTION OF HOOKWORM DISEASE.

In the prevention of diseases caused by animal parasites, we may, of course, attempt to attack the infectious agent in any stage of its life history. In connection with uncinariasis, three periods in particular come into consideration, namely: (1) The adult worm in the intestine; (2) the egg in the feces, and (3) the infecting ("encysted") stage of the larva.

(1) ADULT WORM IN THE INTESTINE; TREATMENT.

The destruction of the adult worm in the intestine not only relieves the patient of an important and (when present in large numbers) serious or even dangerous parasite, but it is also an important factor in preventing the spread of the disease to other people. Accordingly, treatment should be instituted even if the eggs found in the feces are so few in number as to indicate only a light infection.

Not infrequently the opinion is expressed that the infection with parasites found in a given patient is so light that treatment is hardly necessary. Such a view, however, is often very shortsighted, for it is not infrequently light infections occurring at unfavorable seasons and under unfavorable conditions that furnish the material for heavy infections at more favorable times. No *Uncinaria* infection in man is too light to be worthy of treatment, for each adult female may lay eggs; hence the destruction of these females means the decrease of scores of free infectious larvæ.

Not all cases of the malady can be recognized without the microscope; hence many people will unconsciously spread the disease-producing agent. Furthermore, many cases which might be recognized by symptoms will not come under medical treatment, so that they, too, will spread the infectious material. It is clear, therefore, that for satisfactory results in prevention we must adopt some method in addition to the treatment.

(2) EGGS IN THE FECES; CONTROL AND DESTRUCTION.

It is in the feces that we find the potentially infectious material in the most concentrated form. After the eggs develop into embryos the latter may leave the fecal matter and be distributed in the sand or in the water. Accordingly, it is much easier to control or destroy a given amount of infectious matter while it is concentrated in the feces than it is later when it is spread over a larger area. Here, in fact, we have the key to the prevention of uncinariasis. Proper disposal of the fecal discharges will make the spread of uncinariasis impossible. As such proper disposal I will suggest: Properly built privies when sewerage is lacking; use of such outhouses after construction; cleaning the same at regular intervals, and burial, burning, disinfection, or drying of the feces.

(3) THE INFECTING ("ENCYSTED") STAGE OF THE LARVA.

Disinfection of premises.—A chemical disinfection of premises to kill the free stages of eggs, embryos, and larvæ of the parasite would

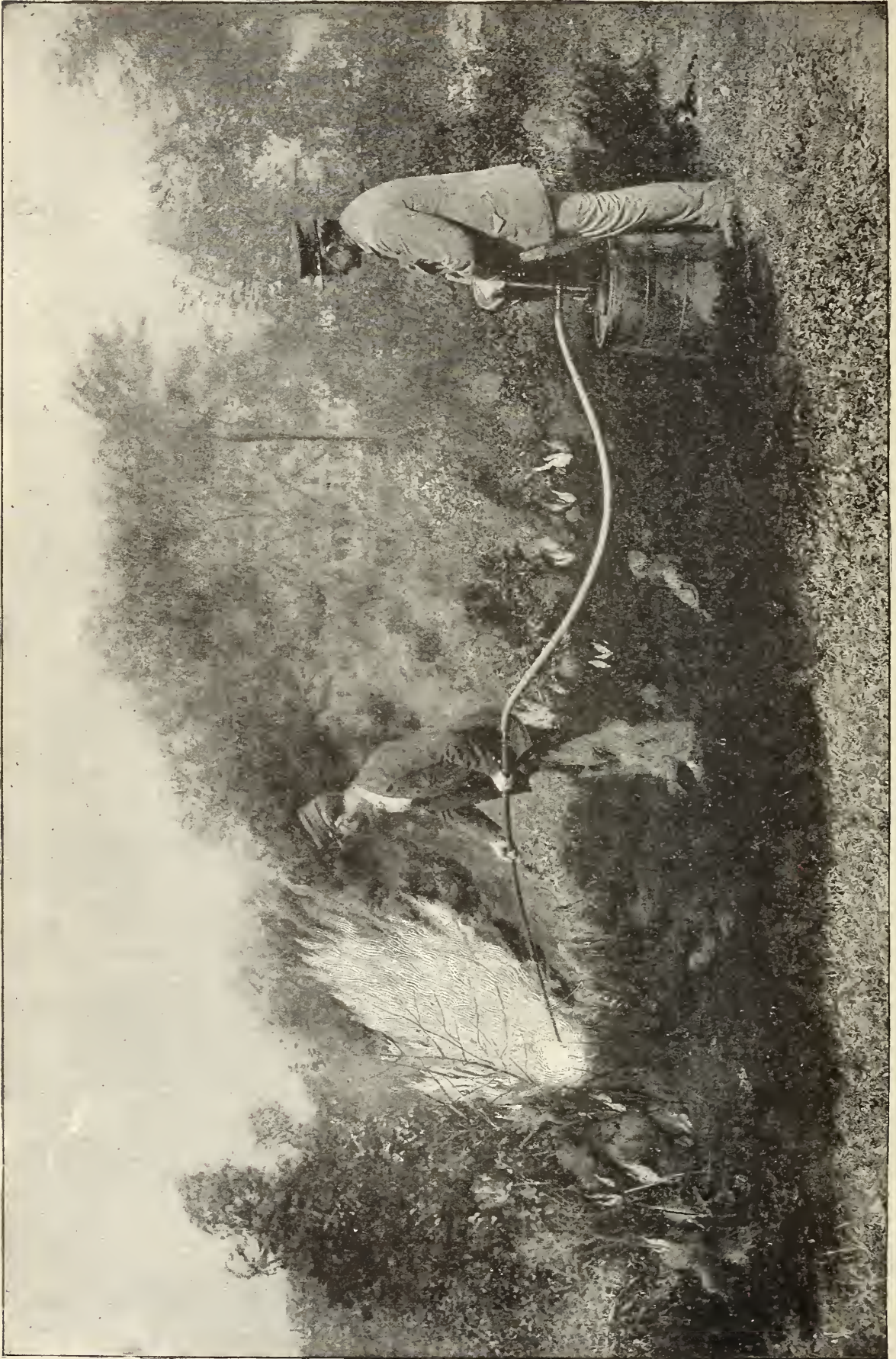


FIG. 86.—Spraying with burning oil, to kill superficial infection on premises where chemical disinfectants can not be used. (After Forbush & Fernald, 1896.)

hardly be practicable, but heat, dryness, and cold all result in killing these organisms.

About twenty-four to forty-eight hours of freezing temperature kills the free infection, hence after any cold weather of this kind in winter it may be assumed that the premises are disinfected.

After any especially dry weather, most if not all the free infection (except such as exists in places not affected by the dryness) is killed, so that exposed portions of premises may be assumed to be practically disinfected.

Spraying with burning oil (fig. 86), as practiced by the Massachusetts Gypsy Moth Commission, will effectually disinfect any area. If a spray nozzle or "cyclone burner" is not at hand, the ground around the house could be strewn with straw or brush and set afire (due precaution being taken not to burn the house), thus thoroughly disinfecting the premises. (See Stiles, 1902 d.)

Drinking water.—To tell the average farm hand or miner that he should always "boil or filter" the water before drinking it is, academically, a step toward preventing infection with uncinariasis. Practically, however, it is a step toward throwing away whatever influence we may happen to have with him. Theoretically, we should teach this simple hygienic precaution to all families, both in the city and in the country. Practically, we are in many cases weakening our position by insisting too generally upon this point.

While, therefore, we may warn people to boil or filter their drinking water in order to prevent the introduction of the infecting agent of uncinariasis or of other diseases, provided we see any chance of their following the advice (in regard to which we ourselves, except in times of epidemics, are very inconsistent), we will, I believe, usually weaken our influence with the poorer classes in mentioning a precaution which the average farm hand naturally looks on as absurd. It is much more important to urge him to locate his privy some distance from the well. That is a proposition he can appreciate: the necessity for boiling or filtering drinking water is usually beyond his mental horizon.

Clean hands.—An important point in connection with preventing the ingestion of the infectious agent of uncinariasis is that the hands and finger nails should be kept clean. I am inclined, however, to take an ultrapRACTICAL view of cleanliness versus dirt in connection with country houses, and to first see that the inevitable dirt shall be clean. This can be accomplished if we can succeed in having properly constructed latrines, built at proper distance from the wells and houses, if the children be taught to use them, and if the parents be taught the necessity for cleaning them.

These, in my opinion, are the first steps to be taken, and far outweigh all such considerations as boiling and filtering drinking water or keeping the hands clean.

Wearing shoes.—Wearing shoes during wet weather and washing the feet frequently will prevent the cutaneous infection and will protect to

a great extent against ground itch. It can hardly be expected, however, that the poorer children in country districts will adopt this precaution to any extent.

COMMON INTERPRETATION OF HOOKWORM DISEASE.

Upon several former occasions I have referred to "cases of anemia of obscure origin" as possibly due to uncinariasis. In a recent paper (Stiles, 1902b, pp. 207-208) I referred to "dirt-eating" as being possibly connected with uncinariasis; it was also intimated (1902b, p. 215), upon authority of Dr. Kirby-Smith, that in Mississippi uncinariasis is confused with malaria; Harris (1902c) also points out that much of the anemia attributed to malaria and dirt-eating is probably due to hookworm disease.

At present I am able to make more specific statements than formerly. The condition which should be attributed to light infections of uncinariasis is usually interpreted as due to malaria or diarrhea; medium cases are usually interpreted as an anemia due to malaria combined with "improper diet" or "insufficient nourishment;" severe cases are usually attributed to "malarial cachexia," "dirt-eating," "resin-chewing," "heart disease," "dropsy," "general debility," "pernicious anemia," and "lack of proper nourishment." Such at least are the most common diagnoses which have been made by the attending physicians in the cases which I have interpreted as light, medium, or severe infections with *Uncinaria americana*.

ECONOMIC IMPORTANCE OF HOOKWORM DISEASE.

Malaria is admittedly one of the most important diseases when viewed from an economic standpoint. In general, uncinariasis is, in the South, fully as important as malaria, and in some respects it is of even greater importance.

Take a given farming area in the sand district with an infection of uncinariasis, and assume that 100 farm hands are employed. It is not an exaggeration to say that these 100 people are not doing the work of 80 or 90 average hands. Thus there is a distinct loss of 10 to 20 per cent in the wages and a corresponding loss in the crop returns. In some places I should estimate the loss at even a higher percentage, say an average of 25 per cent, while in several families which I have examined I should say that uncinariasis is reducing the laboring capacity, hence the productiveness, of the family to as low as 30 to 40 per cent, thus entailing a loss of 60 to 70 per cent.

Nor are the losses in wages and in the laboring capacity, and the decrease of productiveness of the family, hence of the farm, and finally of the county and State, the only economic considerations involved. Cases are not unknown where families have sold, moved, or destroyed their homes, or were about to do so, because of the existence of this disease and because of the belief that it might be due to the locality in which they lived.

Again, it is almost a common experience to be told by the father of a family that he spends for medicine all he earns, in the hope of ridding his children of this malady. Add to this the physicians' bills, the loss by death and funeral expenses, etc., and it is seen that this infection is keeping more than one family in absolute poverty.

Nor should we forget that uncinariasis has its important bearing upon the mental as well as upon the physical and financial development of the poorer white people. As already stated, children infected with this malady are often underdeveloped mentally; frequently they have a reputation in the schools, in the neighborhood, and in their own family, of being "stupid," or "dull," or "backward" in their studies, etc. It has already been mentioned that children suffering with this disease are frequently kept home from school because of their tendency to become edematous when they sit still for any length of time. When we now recall that these conditions coincide especially with the educational period, it should not seem strange that uncinariasis has a marked influence upon the general intellectual condition of the districts in which it occurs.

Considering the subject in the light of all I saw on the trip, and taking what I believe to be a conservative view of the subject, I find it exceedingly difficult to escape the conclusion that in uncinariasis, caused by *Uncinaria americana*, we have a pathologic basis as one of the most important factors in the inferior mental, physical, and financial condition of the poorer classes of the white population of the rural sand and piney wood districts which I visited. This sounds like an extreme statement, but it is based upon extreme facts.

By this position I do not intend to assert that uncinariasis is the only factor which comes into consideration. The warm climate and the monotonous diet, and probably also the excessive use of tobacco in some cases, are not without influence. Still, with uncinariasis as it exists to-day, these people are suffering from a handicap in life which practically removes them from a fair chance in competition. If the uncinariasis is removed they will be placed in a more favorable condition both subjectively and objectively. With the present prevalence of uncinariasis their lack of ambition is perfectly natural; remove the disease and they can develop ambition.

On the other hand, if we were to select the strongest people in the country and place them in the conditions under which these patients are now living it would be only a generation or two before even a race of athletes would be in the same condition as the persons under discussion.

The conditions described are familiar to persons who have visited the rural sand districts. But they have existed for so many years that many of us to-day look upon them as natural, hence they do not attract the consideration to which they are entitled.

GEOGRAPHIC DISTRIBUTION AND ABSTRACTS OF CASES FOUND IN THE UNITED STATES.

In a former paper (Stiles, 1902b, pp. 206-217) I gave abstracts of all the cases of uncinariasis known to me at that time for the United States. The disease is now proved to be so common in certain portions of the country that it is hardly necessary to keep a full record of every case found, but on account of the medico-historical interest associated with the subject, and also in order to complete the literature and details of geographic distribution, there are here added abstracts and notices of various cases which have come to my knowledge since the above-mentioned paper was completed.

NEW ENGLAND STATES.

NEW HAMPSHIRE.

No positively diagnosed cases of hookworm disease seem to be recorded for this State.

Center Eppingham, 1876 ? 1 case, ? death.

GOULD (1876, pp. 417, 418) refers to a case of pica or dirt-eating which sounds suspiciously like uncinariasis.

MIDDLE STATES.

NEW YORK.

Rochester, 1868 ? 1 case, ? death.

ELY (1868, pp. 101, 102) describes a case of chalk-eating which may possibly have been due to uncinariasis, though this is by no means certain.

Buffalo, 1896 5 cases. 0 death.

MÖHLAU'S (1897) cases. See STILES (1902b, p. 209). Doubts have arisen in the minds of some physicians as to whether these were actually cases of uncinariasis.

Glen Island, 1900 4 cases. 0 death.

ASHFORD'S cases from Porto Rico. Probably due to *Uncinaria americana*; reported in STILES (1902b, p. 210).

Stapleton 1 case. 0 death.

BAILHACHE'S, and GREENE'S (1901) case. Place of infection uncertain. See STILES (1902b, p. 215).

Albany, 1900 1 case. 0 death.

WARD (1902, pp. 23-26): American, physician, 32 years old. Had served in U. S. Army in the Philippines. Albany hospital, Feb. 6, 1902. Report on feces by Dr. GEORGE BLUMER, confirmed by Dr. W. S. THAYER.

? Albany, 1900 1 case, 0 death.

NEUMAN and BLUMER. Details of case not known to me.

PENNSYLVANIA.

It is by no means impossible that uncinariasis will be found among the miners of Pennsylvania. As so many of these men are immigrants from Europe, the Old World species, *Agchylostoma duodenale* may be expected.

Philadelphia, 1900-1901 3 cases, 0 death.

BOSTON'S cases reported by ALLYN and BEHREND (1901). See STILES (1902b, p. 211). Probably due to *Uncinaria americana*.

Philadelphia, 1901 1 case, 0 death.

ALLYN and BEHREND'S (1902) case, imported from Italy, hence due to *Agchylostoma duodenale*.

MARYLAND.

Baltimore, 1900 2 cases, 0 death.

HEMMETER'S (1902) cases; probably infected in Porto Rico and due to *Uncinaria americana*. See STILES (1902b, p. 210).

Baltimore, 1901 1 case, 1 death.

HALL'S (1901) case; imported, possibly from Vera Cruz. Due to *Agchylostoma duodenale*. See STILES (1902b, pp. 213-215).

Baltimore, 1902 1 case, 0 death.

OSLER'S case [unpublished]. In Johns Hopkins Hospital. Patient came from North Carolina. Parasites determined by Boggs as *Uncinaria americana*, confirmed by Stiles.

DISTRICT OF COLUMBIA.

Washington, 1901 [1 case, 1 death.]

CLAYTOR'S (1901a, 1902a) case, from Westmoreland County, Va., due to *Uncinaria americana*. See STILES (1902b, pp. 211-212).

Washington, 1902 1 case, 0 death.

HERRICK (1902, p. 101): Male, 37 years old, lived in Germany until 1897. Sent to Philippine Islands September, 1899. "Present illness began in July, 1900, with an attack of diarrhea. He had from 10 to 15 movements daily for three months, with a moderate amount of tenesmus. Mucus and blood appeared in the stools after the first month, giving them a dark tarry appearance. He lost weight and strength rapidly during this time and gradually became short of breath on slightest exertion. This was followed by a period of improvement, the stools becoming less frequent; but in January, 1901, he became worse and was sent to the hospital at Iloilo. Since then he has been confined to hospitals, on account of weakness and dyspnea. In September, 1901, the diarrhea ceased, and although he has gained a little weight he has been steadily growing weaker. At no time had he been subject to hemorrhages other than stated.

"Physical examination shows an apparently well-nourished man with a peculiar lemon-yellow pallor; conjunctivas and mucous membranes are pale; slight edema of the ankles is present. He has marked dyspnea on the slightest exertion. The lungs are negative; the heart is enlarged; the point of maximum impulse is in the fifth intercostal space in the nipple line. A soft blowing systolic murmur is audible at the apex and in the pulmonic area. Liver dullness extends from the sixth intercostal space in the nipple line to 1 cm. below the costal margin. The edge is palpable. The spleen is enlarged and the edge is palpable at the costal margin. The urine is negative.

"The blood is very pale and watery; a moderate poikilocytosis is present: there are no nucleated red corpuscles and no malaria organisms present. The blood count shows: Red corpuscles, 1,120,000; hemoglobin, 18 per cent; leucocytes, about 4,000; polymorphonuclear, 52 per cent; eosinophiles, 26.8 per cent; small mononuclear, 14 per cent; large mononuclear, 4.4 per cent; transitional, 2.8 per cent.

"One month later, the patient meantime having been taking arsenic and iron, the blood count was as follows: Red corpuscles, 1,450,000; hemoglobin, 22 per cent; leucocytes, 2,000; polymorphonuclear, 61 per cent; eosinophile, 18.2 per cent; small mononuclear, 16.4 per cent; large mononuclear, 3.2 per cent; transitional, 1.2 per cent; no nucleated red cells.

"There had been practically no change in the patient's condition. The liver and spleen were as in the former note, and the dyspnoea was marked. Numerous typical ova of the *Uncinaria* were present in the stools, but no adult forms were seen. After the usual thymol treatment about 60 adult worms were found. They resembled in all respects *Uncinaria duodenalis*, and were identified by Dr. Stiles as the Old World hookworm. The ova present were in the progress of segmentation, 4 to 12 cells being visible. None were seen containing an embryo, as frequently occurs in the form *Uncinaria americana*, described by Dr. Stiles.

"Blood examinations, ten and twenty days after the thymol treatment was begun, showed the following counts:

	May 28, 1902.	June 7, 1902.
Red corpuscles.....number..	2,300,000	3,100,000
Hemoglobin.....per cent..	23	27
Leucocytes.....number..	2,500	3,000
Polymorphonuclear.....per cent..	54	62
Eosinophile.....do....	21	14
Small mononuclear.....do....	17	17
Large mononuclear.....do....	6	6
Transitional.....do....	2	1

"The general condition is also improving rapidly, although the parasites are not entirely eliminated, as an ovum is still occasionally found in the stools."

Anacostia (Government Hospital for the Insane), 1902-16 cases, 0 death.

Cases found on microscopic examination by Stiles, Garrison, Ransom, and Stevenson, of United States Public Health and Marine-Hospital Service. Probably most if not all of these were infected in other localities. (See p. 37.)

VIRGINIA.

Essex County, ? date.....: cases. ? deaths.

Passed Asst. Surgeon JOHN F. ANDERSON has stated to me that there exists in Essex County a condition of "bloat" and anemia which is usually attributed to dirt-eating, and which corresponds in general to the conditions described in this paper.

Richmond, 1852.....? 1 case, 0 death.

POLLARD (1852, p. 185) reports a case of dirt-eating. Its connection with uncinariasis is possible, but not clear.

Richmond, 1898.....1 or 2 cases, 0 death.

GRAY's (1901) case. See STILES (1902b, p. 209).

Westmoreland County, 1901.....1 case, 1 death.

CLAYTOR's case. See District of Columbia.

Westmoreland County, ? date.....2 cases, 0 death.

Referred to by STUART in STILES (1901, p. 525, and 1902b, p. 212).

NORTH CAROLINA.

Judging from the size of the eggs, all the cases I found in North Carolina were due to *Uncinaria americana*.

Roanoke River Valley, prior to 1808 ? cases, ? deaths.

PITT (1808) states that malacia or dirt-eating "prevails mostly among the poorer white people and negroes, and originates in my opinion from a deficiency of nourishment." He refers also to the slowly healing ulcers on the legs, and to the "tallow complexion." His general description points quite distinctly to uncinariasis.

Person County, 1832 ? cases, ? deaths.

JORDAN (1832, pp. 18-30) gives a discussion of dirt-eating which quite positively refers, at least in part, to uncinariasis.

Durgy, Person County, 1902 2 cases, 0 death.

STILES (1903b, p. 38).

Cumnock Coal Mines, Chatham County, 1902 1 case, 0 death.

STILES (1903b, p. 38).

Gaston County, about 1880 ? cases, ? deaths.

The following interesting letter has been received from Dr. Barringer, and indicates the presence of uncinariasis in Gaston County:

"DEAR SIR: I have just seen in the Marine-Hospital Service Public Health Reports your letter of October 22, from Kershaw, S. C. I was for many years located in the district in which you have been working, and your letter has thrown an immense amount of retrospective light on what I saw there. My work was done in the early eighties, and yet I still remember many cases of pernicious anemia, which was accompanied in some cases by dropsical effusions and diarrhea, a combination I could never make out, and yet this must have been uncinariasis.

"Whole sections of the illicit distillers of Kings Mountain, in Gaston County, N. C., were affected, and the dirt-eating whites of this section seemed to have a malady different from those of the better class in the neighborhood. I tried a tannic acid preparation, which seemed to do more good than anything else, and I wish now I had tried Areca nut. By the bye, I also recall that my pointer dogs in this section seemed to be afflicted in the same way. During my stay in Gaston County, from 1878 to 1881, I lost two dogs, who used to follow me around to these houses, from an unknown disorder.

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"I remain, yours, very respectfully,

"P. B. BARRINGER, *Chairman*.

"Dr. CH. WARDELL STILES,

"*Cure Marine-Hospital Service, Washington, D. C.*"

SOUTH CAROLINA.

Judging from the measurements of the eggs, all the cases I found in South Carolina were due to *Uncinaria americana*.

Date? ? cases, ? deaths.

HEUSINGER and GEDDINGS, quoted by BLANCHARD, 1888a, could not be traced.

Adams Run, Colleton County, 1902 4 cases, 0 death.

STILES (1903b, p. 41): Orphans at Charleston.

- Barnwell County, 1902** 1 case, 0 death.
 STILES (1903b, p. 41): Medical student at Charleston.
- Berkeley County, 1902** 3 cases, 0 death.
 STILES (1903b, p. 41): Orphans at Charleston.
- Camden, Kershaw County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 39): Brickyard.
- Charleston, Charleston County, 1902** 3 cases, 0 death.
 Dr. DE SAUSSURE, quoted by STILES (1903b, p. 41): Source of infection not stated.
- Charleston County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 41): Medical students at Charleston; came from seacoast islands.
- Charleston, Charleston County, 1902** [15 cases, 0 death.]
 STILES (1903b, p. 41): At orphan asylum. Children came from Dorchester (1), Berkeley (3), Colleton (4), and Charleston (7) counties
- Charleston, Charleston County, 1902** [4 cases, 0 death.]
 STILES (1903b, p. 41): Medical students from Barnwell (1), Florence (1), and Charleston (2) counties.
- Florence, Florence County, 1902** 1 case, 0 death.
 STILES (1903b, p. 41): Medical student at Charleston.
- Lancaster and Kershaw counties, 1902** about 50 cases, 0 death.
 STILES (1903b, pp. 40-41).
- McClellanville, Charleston County, 1902** 4 cases, 0 death.
 STILES (1903b, p. 41): Orphans at Charleston.
- Plum Island, Charleston County, 1902** 3 cases, 0 death.
 STILES (1903b, p. 41): Orphans at Charleston.
- Summerville, Dorchester County, 1902** 1 case, 0 death.
 STILES (1903b, p. 41): Orphan at Charleston.

GEORGIA.

Judging from the size of the eggs, all the cases I found in Georgia were due to *Uncinaria americana*. Harris states that his cases were due to the same species.

Locality? Date? ? cases, ? deaths.

LYELL quoted by BLANCHARD (1888a), could not be traced.

Richmond County, 1836 ? cases, ? deaths.

COTTING (1836a, pp. 288-290) states that clay is eaten by many people, especially by children. Probably at least some of the cases were connected with uncinariasis.

Pine Barrens of Georgia, 1845 ? cases, ? deaths.

LE CONTE (1845, pp. 417-444) states that dirt-eating is common in the pine barrens of Georgia. His description refers quite clearly to uncinariasis, at least in part.

Appling County, 1902 1 case, 1 death.

HARRIS (1902a, pp. 99-100): Male, farmer, 29 years old. Healthy until 14 years of age, then observed that he was never so well in latter part of winter and spring as in summer and fall. Anemic; weak; food tastes salty; in spring the arms, hands, and dorsal surface of feet become greatly inflamed, blisters form, followed by scabs; severe constipation; pains in neck and stomach; vomiting frequent; weight 117 pounds; skin pale and wrinkled, smooth and dry; very little beard; mucous membranes very pale; tongue moist, shows indentations of teeth, and its epithelium in a large measure absent; teeth small, quite a number of them decayed; pulse 90, respiration 20, temperature 98° F.; body somewhat emaciated; heart with soft, blowing systolic murmur constant, varying greatly in intensity; marked venous hum over right jugular; just below the ensiform cartilage and to the left great tenderness; stomach normal in size and position; after Ewald trial meal, total acidity 64, HCl 40, combined HCl 4, phosphates 4; spleen and intestines normal; feces dark brick-red; *Uncinaria* eggs present; urine 2,300 cm. in twenty-four hours, light yellowish-red; specific gravity, 1.012, faintly alkaline, no sugar, at one time faint ring of albumin, albumose not present; urea in twenty-four hours, 21.15 grams, uric acid 0.475 gram, chlorids 3.15, phosphates 2.37, sulphates 2.82 grams. Blood: Red corpuscles 1,760,000, white 4,020, hemoglobin 20 per cent; decided though not extreme poikilocytosis, a number of microcytes; a few nucleated reds; small lymphocytes 28, large lymphocytes 14, transitional 6, polymorphonuclear leucocytes 50, eosinophiles 2. Vision, right eye 15-20, left eye 15-30. Diagnosis: Anchylostomiasis and possibly pellagra. Treatment: Afternoon, 10 grains of calomel; next day, 7 a. m., 30 grains of thymol in capsules; 9 a. m., 30 grains of thymol; 8 p. m., large dose of salts. Stools contained at least 420 worms.—HARRIS, 1902b, pp. 220-227. Same case.

Porter Springs 4 cases, 0 death.

Letter of H. F. HARRIS, dated August 9, 1902, to U. S. Bureau of Animal Industry. He states: "I am absolutely sure that this disease is very common in all this region." One of the four cases probably originated in Troup County, the other three in Lumpkin or neighboring county.

Locality ?, 1902 7 cases, 0 death.

HARRIS (1902c, p. 776) states that since reporting his first case he has discovered eleven new cases for Georgia. See also Porter Springs.

Atlanta, 1902 1 case, 1 death.

CLAUDE A. SMITH (1902, p. 1062): Case reported; mentioned also a similar case in a dog which had eaten some of the infected feces of the patient. Man died of pleuritic abscess. The specimens were collected post-mortem in a negro at Grady Hospital, Atlanta.

Dr. Smith kindly sent me the parasites for examination. The specimens from man (B. A. I., No. 3423) proved to be *Uncinaria americana*, while those from the dog (B. A. I., Nos. 3424 and 3425) were *Ancylostoma caninum*.

Atlanta, November, 1902 2 cases, 0 death.

CLAUDE A. SMITH informed me in a personal letter dated December 3, 1902, that he had just observed two cases, both from Florida. One was a man 50 years old, the other a dental student.

Albany, Dougherty County, 1902 about 5 cases, 0 death.

STILES (1903b, p. 43): People did not belong in Albany.

Americus, Sumter County, 1902 1 case, 0 death.

STILES (1903b, p. 41): Orphan in Macon.

- Baxley, Appling County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Buena Vista, Marion County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 41): Orphan in Macon.
- Cordele, Dooly County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Darien, McIntosh County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Effingham County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Fort Valley, Houston County, 1902** 50 cases, 0 death.
 STILES (1903b, p. 42): Dr. BROWN stated he could easily find 50 or more cases. We examined about 10 cases together.
- Jackson County, 1902**? cases, ? deaths.
 Dr. HARDMAN, quoted by STILES (1903b, p. 41).
- Johnson County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Jones County, 1902** 4 cases, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Kinderlou Station, Lowndes County** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Lee County, 1902** 4 cases, 0 death.
 STILES (1903b, p. 43): Observed with Dr. HILSMAN.
- Lyon, Tattnall County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Macon, Bibb County, 1902**[29 cases, 0 death.]
 STILES (1903b, pp. 41-42): At orphan asylums. See Americus (1), Baxley (1), Buena Vista (1), Cordele (1), Darien (1), Effingham (1), Johnson (1), Jones (4), Kinderlou (1), Lyon (1), Monroe (1), Richwood (2), Sandersville (1), Thomasville (1), Savannah (1), Waycross (3), Monroe (1), Georgia. Also: Deland (4), Liveoak (1), Wacissa (1), Florida.
- Macon, Bibb County, 1902** about 25 to 30 cases, 0 death.
 STILES (1903b, p. 42): About 25 to 30 cases; men, women, and children in the cotton-mill families.
- Monroe County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Richwood, Dooly County, 1902** 2 cases, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.
- Sandersville, Washington County, 1902** 1 case, 0 death.
 STILES (1903b, p. 42): Orphan in Macon.

Savannah, Chatham County, 1902 1 case, 0 death.

STILES (1903b, p. 42): Orphan in Macon.

Thomasville, Thomas County, 1902 1 case, 0 death.

STILES (1903b, p. 41): Orphan in Macon.

Washington County, 1902 ? cases, 0 death.

In personal conversation with Dr. A. MOODY BURT, I was informed that there were a number of persons in Washington County who show in general the symptoms of uncinariasis.

Waycross, Ware County, 1902 3 cases, 0 death.

STILES (1903b, p. 42): Orphan in Macon.

Waycross, Ware County, 1902 ? cases, ? deaths.

STILES (1903b, p. 43): Many cases, number not estimated; about 20 cases of uncinariasis to 1 of malaria; on authority of Drs. IZLAR and WALKER.

Willacoochee and vicinity, Coffee County, 1902 200 cases, 0 death.

STILES (1903b, p. 43): Given on authority of Dr. WILCOX. We examined 8 cases together.

FLORIDA.

Judging from the size of the eggs all the cases I found in Florida, so far as examined microscopically, were due to *Uncinaria americana*.

Prior to 1845 ? cases, ? deaths.

LITTLE (1845) refers to dirt-eating; see STILES (1902b, p. 208).

Locality ?, 1902 ? cases, ? deaths.

GUI TERAS's cases; see STILES (1902b, p. 215).

Locality ?, 1902 1 case, 0 death.

HARRIS (1902c, p. 776): Locality not given; originated in Florida.

Baker County ? cases, ? deaths.

In personal conversation with a Jacksonville druggist, I was informed that so-called dirt-eaters are common in Baker County.

Clay County ? cases, ? deaths.

In personal conversation with a Jacksonville druggist, I was informed that so-called dirt-eaters are common in Clay County.

Deland, Volusia County, 1902 4 cases, 0 death.

STILES (1903b, p. 42): Orphans in Macon, Ga.

Jacksonville, Duval County, 1902 2 cases, 0 death.

STILES (1903b, p. 44).

Liveoak, Suwanee County, 1902 1 case, 0 death.

STILES (1903b, p. 42): Orphan in Macon, Ga.

Tampa, Hillsboro County, 1903 12 cases, 0 death.

In a letter dated March 21, 1903, Dr. J. S. HELMS says: "I have to date collected 12 cases and am yet working. I dare say that there are hundreds of cases in south Florida." The parasites were *Uncinaria americana*.

Wacissa, Jefferson County, 1902-----1 case, 0 death.

STILES (1903b, p. 42): Orphan in Macon, Ga.

Ocala, Marion County, 1902-----5 cases, 0 death.

STILES (1903b, p. 44).

Twiggs County, 1902-----? cases, ? deaths.

I have been informed that in Twiggs County there exists a condition which corresponds to uncinariasis.

Upson County-----? cases, ? deaths.

There is said to exist a great deal of "bloat" in this county. Possibly this "bloat" is due, in part at least, to uncinariasis.

Waldo, Alachua County, 1902-----about 12 cases, 0 death.

STILES (1903b, p. 44).

ALABAMA.

? Locality-----? cases, ? deaths.

LYELL, quoted by BLANCHARD, 1888a. Could not be traced.

Middle Alabama, 1902-----1 case, 0 death.

HARRIS (1902c, p. 776).

Mobile and vicinity-----24 cases, 0 death.

The following extracts are made from a letter dated March 3, 1903, from DR. E. D. BONDURANT, professor of pathology, medical department, University of Alabama: "Some weeks ago a fellow practitioner told of cases of intense and protracted anemia he was treating, suggested the possibility of uncinariasis, and asked me to make a microscopic examination of the fecal discharges. This was done, and I had no difficulty in promptly identifying the hookworm ova in the feces of every one of his 4 cases. Shortly afterward I came upon 2 cases in my own practice, found the ova in quantity, and after thymol I found numbers of adult worms. At the city hospital we have already had several [?] others, and one physician who has actively taken up the search in his anemic country patients tells me that he has found about 15 cases. There is no doubt that the disease is *very common* in the country surrounding Mobile. * * * Our 'poor whites' are surely widely infected with the disorder * * *."

In a letter dated March 13, Dr. E. D. Bondurant says: "I have, since I last wrote you, diagnosed cases from Monroe County, Covington County, and Crenshaw County, this State, as well as numerous other cases in the district immediately around Mobile. * * * All of my cases have promptly improved after thymol."

Monroe County, 1903-----1 case, 0 death.

Quoted on the authority of a letter dated March 3, 1903, from DR. E. D. BONDURANT.

Waldo, Talladega County-----? cases, ? deaths.

A trained nurse, Miss Edith Lide, has described to me a family at Waldo whose symptoms (anemia, heart, emaciation, dirt-eating, etc.) point almost unmistakably to hookworm disease.

MISSISSIPPI.

One of the American physicians (Dr. Kirby-Smith) who saw several cases of uncinariasis in Cuba has recently stated to me that this disease is undoubtedly present in Mississippi, but its exact nature has not been recognized. He is convinced that he himself has seen a number of cases which were confused with malaria.

ARKANSAS.

Uncinariasis does not appear to be proved as yet for Arkansas. but I have been told that it is probably present.

LOUISIANA.

Louisiana, prior to 1821 and 1850 ? cases, ? deaths.

CHABERT'S (1821a) and DUNCAN'S (1850, St. Mary Parish) accounts of dirt-eating apply very well to uncinariasis. See STILES (1902b, p. 207).

New Orleans, 1899 1 case, 0 death.

TEBAULT'S (1899) case. See STILES (1902b, p. 209).

Dirt-eaters are said to be numerous in the Mississippi Delta near Baton Rouge.

TEXAS.

Locality?, 1864 1 case, 1 death.

HERFF'S (1864) case in Mexican woman. See STILES (1902b, p. 208).

Galveston, 1894 1 case, 0 death.

ALLEN J. SMITH'S case, reported by SCHAEFER (1901). See STILES (1902b, p. 208).

Galveston, 1900-1901 1 case, 0 death.

SCHAEFER'S (1901) case; probably infected in Mexico. See STILES (1902b, p. 211).

Galveston, 1901 8 cases, 0 death.

ALLEN J. SMITH'S cases, reported in part by SCHAEFER (1901). See STILES (1902b, p. 211). At least one of these cases was due to *Uncinaria americana*.

Encinal, La Salle County; Heampstead, Waller County; Lavaca County, 1898 ? cases, ? deaths.

Upon seeing my description of hookworm disease and the photograph from which figure 42 was made, MR. CHARLES A. PFENDER, assistant in the Zoological Laboratory, U. S. Bureau of Animal Industry, stated to me that he had seen similar conditions among Mexican children in Encinal, among negroes at Heampstead in Brazos River bottom, and among the poorer people in the southern portion of Lavaca County, along the Navidad River.

CENTRAL STATES.

ILLINOIS.

Chicago, 1902 1 case, 1 death.

CAPPS (1902a; 1903a, pp. 28-33): Patient, G. L., in Cook County Hospital; male; 52 years old; carpenter; American. Infection probably took place at Panama. Earliest symptoms two years ago, aching pain in upper belly, loss of appetite, and weakness. No nausea or vomiting. Bowels irregular, loose or constipated. Later, shortness of breath and dizziness; also palpitation of heart. In hospital fifteen months at New Orleans; diagnosis, pernicious anemia. September 9, 1902, admitted to Cook County Hospital; pains in belly, weakness, dyspnea, and palpitation. After sitting or standing feet would swell. Frequent dizziness and faintness. Occasionally had developed moderate fever, at which time epigastric pain was worse. Appetite poor. Constipation. Gradual, moderate emaciation.

"*Status presens*.—Man of medium build; skin of a lemon-yellow color. Fatty layer fairly well preserved. Sclerotics bluish and muddy. Lips and mucous membranes almost bloodless. Palpable arteries moderately thickened. Pulse of large volume, soft and compressible; low tension, with a decided water-hammer character. Lungs negative. Heart dullness extended to the left nipple line, to the upper border of the third rib above, and to the right edge of the sternum. The impulse was forcible and diffuse. Over the apex was a systolic blow transmitted to the anterior axil-

lary line. A louder bruit of a different pitch was audible over the mitral area and the base. The pulmonic sound was louder than the aortic closure.

"The spleen was not palpable, though its area of dullness was enlarged. The liver was not felt. The epigastric and umbilical regions were tender to pressure. Knee jerks present but not prompt.

"Temperature 98.6, pulse 90, respiration 20. Urine 1.015; no albumen, no sugar, and no casts.

"September 11, blood examination showed hemaglobin 18 per cent; reds, 2,576,000; whites, 6,600. No stained preparations were made.

"September 15, gastric contents were expressed one hour after a test meal of tea and toast. No free HCl present; no lactic acid.

"September 23, blood examination, hemog. 17 per cent; reds, 2,280,000; whites, 6,000.

"October 20, blood examination, hemog. 12.5 per cent; reds, 843,000; hematocrit, 980,000; whites, 4,500; color index, 0.80; volume index, 1.17. The differential count showed: small mononuclear, 10 per cent; large mononuclear, 11 per cent; polymorph. neutrophile, 66 per cent; polymorph. eosinophiles, 13 per cent; no rouleaux formation; no nucleated red cells; poikilocytosis marked; polychromatophilia marked.

"The presence of pronounced eosinophilia in a case of grave anemia made us strongly suspect the existence of an intestinal parasite, so that the stools were examined frequently. The earlier specimens of feces were watery from the rectal injections employed, and were therefore not easily studied. In a formed movement, however, the ova were found in large numbers. These eggs corresponded accurately in dimensions to those of *Uncinaria duodenalis*, measuring about 56 microns in length and 34 microns in width.

"Subsequently the eggs of *Tricocephalus dispar* were found in small number by Dr. J. L. Miller. Charcot-Leyden crystals were present in some preparations, absent in others. Cover glass smears of feces hardened in alcohol and ether were stained with hematoxylin and eosin and eosinophilic granulations demonstrated. These granulations, like the Charcot crystals, were never numerous, as is so often the case in ankylostomiasis.

"The eggs were successfully cultivated and the larvæ brought to mature development. These experiments will be described later.

"November 6, examination of the blood gave: hemaglobin 11 per cent; reds, 748,000; hematocrit, 915,000; whites, 5,600; color index, 88 per cent; volume index, 122 per cent. Differential count: small mononuclear, 15.8 per cent; large mononuclear, 6.8 per cent; polymorph. neutrophile, 70.2 per cent; eosinophile, 7.6 per cent; eosinophilic myelocytes, 0.2 per cent; mast cells, 0.4 per cent. Poikilocytosis and polychromatophilia marked. Coagulation time, five minutes.

"Previous to this examination the patient had taken thymol in small doses, and it is not unlikely that many parasites were swept away and lost in the stools. The diminished eosinophilia and the small number of eggs found in the stools thereafter lend support to this assumption.

"*History in the hospital.*—Treatment, on the whole, was unsatisfactory, because of the profound weakness of the patient and the irritable condition of the stomach. A persistent nausea set in that interfered with stomach feeding and made the administration of thymol ineffective and even hazardous. Nutrient and salt enemata were resorted to, but the vomiting persisted until the patient succumbed, on November 13.

"During his stay in the hospital the most conspicuous symptom was epigastric pain of a dull nature at first, subsequently colicky. In the last two weeks this pain became continuous, and was accompanied by a great tenderness over the epigastric and right hypochondriac regions. The liver mass extended at this time about 2 inches below the costal arch, and the tenderness, on pressure, was as great as that seen in hepatic abscess.

"The bowels, as a rule, were constipated, and required rectal enemata. The tem-

perature was usually normal, and ranged between 98.5° and 99.5° F. A tendency to hemorrhage was nowhere to be seen, except from the intestinal tract. The feces gave the prussian-blue reaction for iron.

"The examination of the eye grounds was twice carried out under difficulties. No retinal hemorrhage was apparent.

"The blood findings throughout were of a most suggestive nature; an anemia, at first of the secondary type, progressing until it corresponded in most respects to a primary pernicious anemia. The individual corpuscles grew larger and held an ever-increasing amount of hemoglobin, the color index rising from 38 to 88 per cent, and the volume index reaching 122 per cent. The poikilocytosis was sufficiently outspoken, as well as the polychromatophilia, for a primary anemia. The scarcity of nucleated red corpuscles and the entire absence of megaloblasts is unusual in the primary pernicious form, yet some such cases have been reported. What convinced us that the anemia was not of the usual primary type was the eosinophilia of 13 per cent, for in the primary disease the eosinophiles are rarely increased. On the other hand, the *Uncinariae* and most of the other intestinal parasites are characterized by an increase in the eosinophilic cells.

"Autopsy.—The post-mortem examination was made November 13 by Dr. Harris, resident pathologist of the hospital. His report is as follows:

"Body is that of a fairly well-developed and fairly well-nourished man 162 cm. tall. Post-mortem rigidity and lividity present.

"There is a scar 0.5 cm. long on left arm, some edema of lower extremities. Panculus adiposus well preserved and of a light-yellow color.

"Abdominal cavity: Diaphragm reaches to the fifth rib on the right side and to the sixth rib on the left. Some free straw-colored fluid in peritoneal cavity. Omentum extends down to the pelvis over the intestines and contains a considerable quantity of fat.

"Pleural cavities: Some firm fibrous adhesions at right apex. About 1,000 c. c. straw-colored fluid in right pleural cavity; about 250 c. c. in left. Lungs do not meet in median line.

"Pericardial cavity: About 500 c. c. of straw-colored fluid in the pericardial cavity. This fluid contains a few fibrinous flocculi. The pericardium is thin and surface is smooth.

"Tongue, pharynx, larynx, not examined. Thymus absent. Esophagus and trachea negative.

"Lungs: Left lung smooth externally, marginal emphysema, crepitates throughout, floats in water, cut surface smooth, pale, and drips fluid. On the diaphragmatic surface is a caseated nodule 8 mm. in diameter. Right lung with the exception of the caseous nodule corresponds to the description of the left. Both lungs are quite free of pigment and weigh 2,870 grams. Peribronchial lymph glands are negative except for anthracosis.

"Heart: Cavities are quite empty, left ventricle contracted; aortic and pulmonary semilunar valves are competent, read by water test. Some fibrous thickening at the base of the aortic valves. One of the valves has small fenestration 3 mm. long. Pulmonary valves show no change. The mitral orifice admits three fingers, and the valve shows a small amount of fibrous thickening, especially near the free borders. The tricuspid orifice admits five fingers; the valves show no changes. The left ventricle has an aberrant corda tendina extending from the septum to the left wall. Ventricular wall measures 16 mm.; right ventricular wall measures 5 mm. Heart muscle is firm and very yellow, but not mottled. Heart weight, 300 grams. There is a slight increase of the subepicardial fat. There are a few atheromatous patches in the ascending aorta.

"Spleen: About one-half larger than normal; capsule is smooth, parenchyma is quite firm; malpighian bodies prominent; there is an evident increase of connective tissue. The organ weighs 225 grams.

"Kidneys: Right weighs 175 grams. Section pale, cortical markings not well seen; relation between cortex and medulla is normal; capsule strips readily; pelvis is normal. Left kidney weighs 135 grams. Answers to the description of its fellow.

"Ureters, bladder, testicles, and adrenals present no abnormal appearances.

"Liver: Extends 4 cm. below costal arch; capsule smooth and glistening—presents yellowish mottled appearance. Cut surface mottled yellow and red, lobules well seen. Liver cuts with decreased resistance. Weighs 1,600 grams. Bile ducts patent, bile dark brown, no concretions. Pancreas shows no changes.

"Stomach: Of normal size, externally is normal; mucosa is covered with much grayish-yellow mucus; no parasites.

"Small intestines: External appearance normal. Intestines contain a very large amount of very tenacious mucus. In the duodenum was found one hook worm. One hundred and fifty were found in the jejunum and upper part of ileum, being most numerous in the upper and middle jejunum. None found in last 18 inches of ileum. They were very adherent, and were in the proportion of about four females to one male. At point of attachment of some of the parasites was found a small ecchymotic spot, and scattered along the rest of the mucosa a few other ecchymotic spots were seen—possibly points of previous attachment. The mucus in places was blood-stained. Mesenteric glands were enlarged and of a pinkish color. Appendix lies in false pelvis to the outside of psoas muscle and behind cecum—bound down by fibrous adhesions and curled at its tip.

"Spinal cord shows no microscopic changes.

"Bone marrow removed from femur. Marrow is yellow and very fatty. At two points it had a reddish color, but was even here practically all fat.

"*Anatomic diagnosis:* 1. *Uncinaria duodenalis* of small intestine. 2. Ecchymosis of intestinal mucosa, and hemorrhage into intestinal lumen. 3. General anemia and edema of dependent portions of body. 4. Bilateral hydrothorax. 5. Hydropericardium. 6. Edema of lungs and caseous tuberculosis of lower left lobe. 7. Slight atheroma of aorta. 8. Fatty degeneration of heart and liver. 9. Aberrant corda tendina. 10. Passive congestion and fatty degeneration of liver. 11. Chronic interstitial splenitis."

WESTERN STATES.

MISSOURI.

St. Louis, 1893.....1 case, 0 death.

BLICKHAHN's (1893a) case; probably imported from Germany and caused by *Agchylostoma duodenale*. See STILES (1902b, p. 208).

St. Louis, 1901.....1 case, 0 death.

DYER's (1901) case. See STILES (1902b, p. 213).

CALIFORNIA.

San Francisco, 1902.....2 cases, 0 death.

Letters from Drs. G. H. EVANS and MARY HALTON, 1902: Two soldiers who returned from the Philippines. Through the kindness of the observers I was able to examine specimens from one of these cases, and to convince myself that they belonged to the American species. The previous history of the patient was not obtained.

San Francisco, 1903.....2 cases, 0 death.

BROWN (1903, p. 107): Three patients infected with *Strongyloides*; 2 of these (natives of Porto Rico) were also infected with *Uncinaria*.

? Locality.....3 cases, 0 death.

L. MIFFITT's (cases cited by CAPPS, 1903a). Two cases from Mexico, one from the Philippines. Diagnosis by ova.

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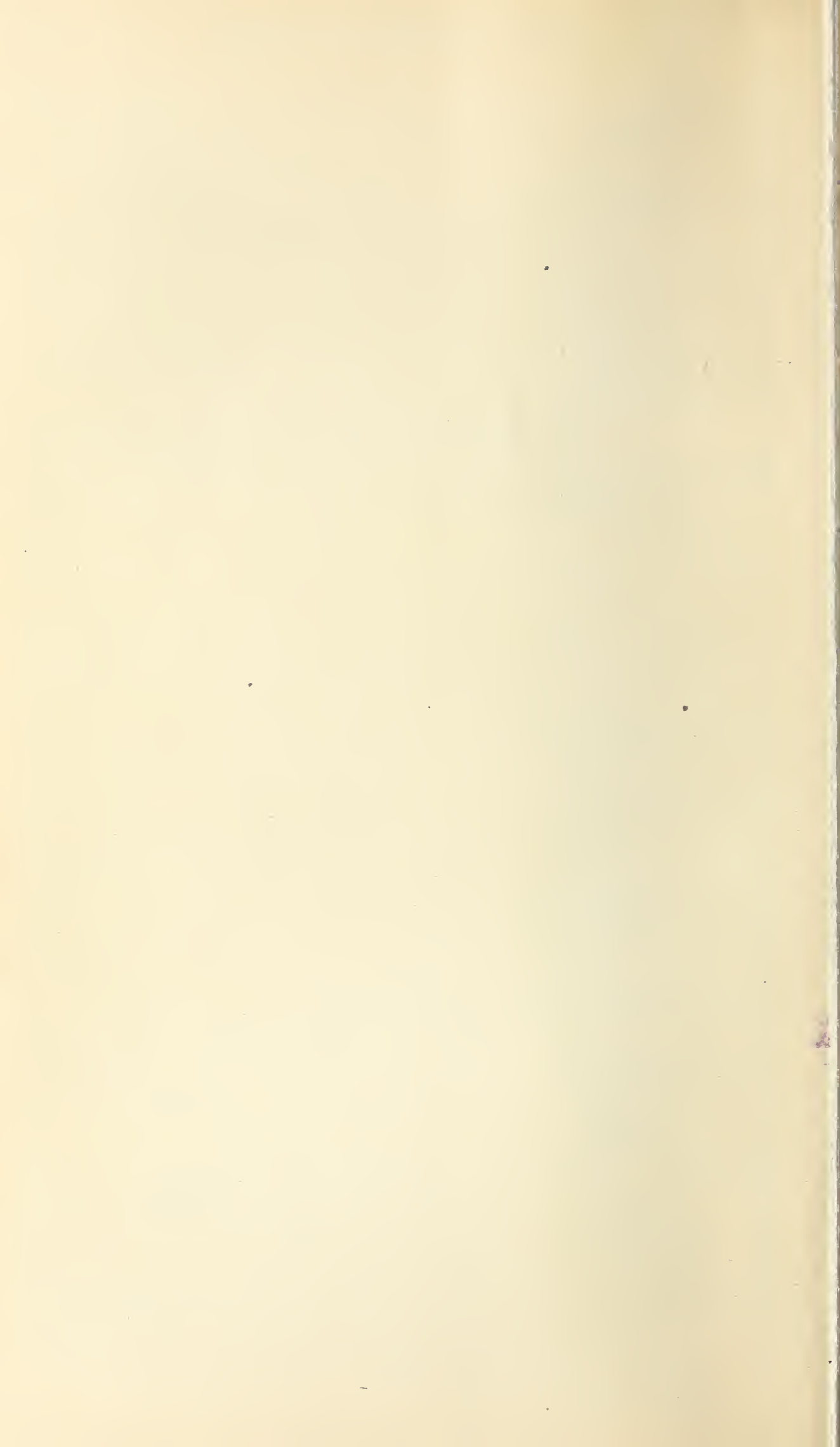
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TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

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AN EXPERIMENTAL INVESTIGATION OF TRYPANOSOMA LEWISI.

BY

EDWARD FRANCIS.



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AN EXPERIMENTAL INVESTIGATION OF TRYPANOSOMA LEWISI.

By EDWARD FRANCIS.

Assistant Surgeon, U. S. Public Health and Marine-Hospital Service.

The great importance of a thorough familiarity with trypanosomes is seen when we consider that within the past year and a half the recognition of trypanosomes was forced upon our governmental medical officers in the Philippines, where the army horses and mules were dying in great numbers, due to the presence in their blood of a parasite which the untrained eye might readily regard as a spirillum or a filaria, but which proved to be the trypanosome of Surra (1). It is probably best in the beginning to say something of trypanosomes in general. They are animal parasites (Hematozoá) of large size which are often found in enormous numbers free in the blood plasma, but do not invade the interior of the blood corpuscles. They manifest a strikingly vigorous eel-like motility as they dart among the corpuscles, pushing them aside.

The *Trypanosoma Evansi* is the cause of surra, a fatal disease of horses and mules in India and the Philippines.

The *Trypanosoma Brucei* causes the tsetse fly disease, or Nagana, which attacks the horses and cattle in central Africa.

The *Trypanosoma equinum* is the cause of mal de caderas, a disease of horses, etc., in South America.

Dourine, or *maladie du Coût*, a disease of horses and dogs, particularly in Algeria and Spain, has been attributed to the *Trypanosoma equiperdum*.

The *Trypanosoma Lewisi* is the cause of a nonfatal affection of wild rats, which harbor spontaneously the parasites in enormous numbers in their blood plasma.

And now within the past year we have a trypanosome of man.

Whether the six trypanosomes mentioned above represent really distinct species or whether two or more of them will be found to be identical must remain for further scientific investigation. Morphologically all six have many structures in common, but they also show

certain differences. Still, an accurate differential diagnosis on the basis of structure alone is not altogether practicable.

As a harmless inhabitant of the blood still other trypanosomes are harbored by the hamster, certain fish, frogs, and birds.

OCCURRENCE AND MORPHOLOGY OF *TRYPANOSOMA LEWISI*.

Our studies were made entirely with the rat trypanosome (*Trypanosoma Lewisi*). The natural host is the wild gray rat and the sewer rat. These rats harbor the trypanosomes in their blood and they infect each other spontaneously.

Crookshank (2) investigated a number of rats in London and found that 25 per cent of the apparently healthy rats harbored trypanosomes.

Rabinowitsch and Kempner (3) examined the blood of fifty wild rats caught about the city of Berlin and found that eighteen of them were infected with trypanosomes.

Laveran and Mesnil (4) found two cases of infection among forty-three sewer rats examined in Paris.

We examined sixty of our home rats caught in different parts of Washington and found no cases of infection.

Natural infection of the white or spotted rats has never yet been found, although they are very susceptible to the infection by inoculation. We inoculated the trypanosome into white rats and examined the blood in fresh and stained preparations.

The parasite may be considered in three parts: The body, the undulating membrane, and the flagellum. The body is the elongated portion which has attached to its side the undulating membrane and to one of its ends the flagellum. The body of the adult parasite measures 13 to 25 micra in length, which is about two to three times the diameter of a red blood corpuscle. Its breadth is 2 to 4 micra. The body runs to a beak-like process at one end, while at the other end there is a free flagellum 7 to 15 micra in length. The flagellate end is considered the anterior or front end because locomotion takes place in the direction of the flagellum, while the beak end is designated the hind end, since it follows the body in locomotion. In the hinder fourth of the body, about 4 micra from the end, there is a short rod-shaped structure, the long axis of which is at a right angle to the long axis of the body; this spot is called the centrosome. It can with difficulty be seen in a fresh preparation. In the anterior fourth of the body of the parasite there is a larger strongly refractile spot, which is regarded as the nucleus.

The undulating membrane is a clear, fine, transparent membrane which presents an attached border, a free border, and a web. It takes its rise from the centrosome, and is fixed by its attached border along the length of the body of the parasite and terminates at the front end, while the outer free margin of the membrane is thickened into a rod-like border which is continued past the front end into the long free-

flagellum. Thus the thick border of the undulating membrane is continuous behind with the centrosome and in front with the flagellum. The web of the membrane is difficult to stain and is seen only faintly in stained preparations. In a fresh preparation the body, the undulating membrane, and the flagellum appear to consist of a homogeneous, strongly refractile protoplasmic substance possessing marked contractility.

A disputed point is the biological relation of centrosome to nucleus. Rabinowitsch and Kempner, after a study of numerous stained and unstained preparations, especially of the developmental forms of the trypanosomes, and after an investigation of the views held in regard to the nucleus of the flagellata, came to the conclusion that the centrosome and nucleus are an interdependent whole which corresponds to the nucleus of the other flagellata. They maintain that in the early stage of development of the parasite the centrosome and nucleus represent a whole which, as the parasite becomes older, breaks into two parts which pass to either end of the adult parasite. They consider the trypanosome's nucleus as made up of two parts more or less separated; the small oval spot situated in the hind end of the trypanosome they designate the nucleolus, while the larger structure found in the front end they call the chromatin heap.

Wasielewski and Senn (5) think that the centrosome is in no way whatever connected with the nucleus. They consider the trypanosome as made up of two parts, the "plasma" and the "periplast." The plasma is the body of the parasite containing the nucleus. The periplast is the outer covering of the trypanosome and embraces the centrosome, undulating membrane, flagellum, and an outer coat investing the body of the parasite. According to this view the centrosome is intimately connected with the undulating membrane. It is the root from which the thickened free border of the undulating membrane springs and has nothing to do with a nucleolus, as Rabinowitsch and Kempner point out, nor with a micro-nucleus, as held by Plimmer and Bradford. In their illustrations the outer coat of the parasite takes a different stain from the body plasma.

Laveran and Mesnil mention that blood kept some time shows trypanosomes reduced to centrosome and flagellum, and that the continuity of the centrosome and flagellum can be seen. We have seen the same thing in stained preparations of fresh blood.

CYCLE OF DEVELOPMENT.

We kept fresh blood in hanging drop at room temperature and at 37° C. and watched it for hours without seeing any of the parasites advance in development beyond the stage which they presented when the blood was first drawn. The different stages of development can be seen only with the help of animal experiments. It is very doubtful if multiplication of the organisms occurs outside the body.

Rabinowitsch and Kempner were the first to employ white rats inoculated intraperitoneally for the study of development, and they were able to trace the entire cycle of development by the examination at short intervals of the blood of a white rat so infected. By a similar examination of a great many rats we were able to get all the forms of development.

Multiplication by transverse division.—The first thing to be observed is a change in the outlines of the trypanosome. Its sharp beak becomes blunt; the flagellate end is no longer sharp, but rounded; the slender body becomes thickened and swollen; both the longitudinal and the transverse diameters of the parasite are increased. There is a multiplication of nuclei and centrosomes to a number which seldom exceeds five each, and they are arranged in a line parallel to the long axis of the parent trypanosome. After the division of nuclei and centrosomes, the next step is the formation of the new flagella, each of which takes its origin in a centrosome and generally emerges from the parent trypanosome on the side which bears the undulating membrane (Pl. III, fig. 9). These new flagella gradually attain full length. The old flagellum becomes destroyed and disappears. The cleavage of the protoplasm of the parent occurs along lines at right angles to the long axis, so that each new segment of protoplasm is equipped with a nucleus, a centrosome, and a flagellum.

The daughter cells are soon free in the blood and are readily distinguished from the adults by their small size and oval outline. They, however, gradually lengthen out into the familiar picture of the adult.

Multiplication by longitudinal division.—This form of multiplication has much in common with the transverse division. There is the same change in outline of the parent and the same multiplication of centrosomes and nuclei to a number of from two to six. The arrangement of the latter, instead of being in a line parallel to the long axis of the parent, is in a transverse line. The new flagella next appear. They arise at the front end of the parent and are arranged closely about the old flagellum and correspond in number to the centrosomes. (Pl. I, fig. 3.) Rabinowitsch and Kempner, in speaking of the origin of the new flagella, mention that in the flagellata the formation of the new flagella takes place so rapidly that the investigators can not follow the process accurately in all its details. They do not hold to the view that flagella multiply by a cleavage in the long axis of the old flagellum. It is their opinion that the new flagella arise from the body plasma and their principal support for such an origin is that in transverse division the new formation of flagella does not occur at the end close about the old flagellum, but on the side at a considerable distance away from the old flagellum.

Multiplication by segmentation.—These three forms of division are separated only for description. They may all be seen side by side in the same preparation and in some instances the distinction between them is not altogether clear, especially between the longitudinal and

transverse modes. Division by segmentation begins with a curving of the parasite which continues until the two ends approach each other and finally meet, thus giving the parasite a globular form. The flagellum becomes lost and the undulating membrane disappears. The nuclear multiplication now occurs and proceeds in the same manner as in the longitudinal and transverse division. The nucleus becomes swollen, appears coarsely granular, and divides into two parts, each of which probably divides and subdivides.

In stained preparations Rabinowitsch and Kempner saw small bodies in the new nuclei. These bodies, which were about the size of centrosomes, took the stain deeply and were placed at the periphery of the nucleus. Whether centrosomes can arise from nuclei or only from other centrosomes is a point of controversy and is mentioned under morphology.

Whatever may be the source of the centrosomes their number always keeps pace with the number of nuclei. No matter how many are formed there is a formation of a corresponding number of centrosomes until in the end there are as many centrosomes as nuclei in the trypanosome.

As regards the manner of the arrangement of nuclei and centrosomes in the globular parent, we find them in two concentric circles. The outer circle contains the nuclei and the inner circle the centrosomes. In the cleavage of the protoplasm there are as many segments as number of nuclei. While in transverse and longitudinal division there are not more than 6 daughter cells, we find in this form as many as 16. Plate III, figure 12, shows 14 segments.

Cleavage proceeds from the periphery to the center of the mass, so that a rosette arises as in the segmentation of malaria. Each segment of protoplasm contains a centrosome and a nucleus, and a flagellum develops from each. In hanging drop these rosettes are seen to be in agitation as soon as they acquire their flagella. Finally the segmentation is complete and the daughter trypanosomes are seen as small oval young forms free in the blood. Rabinowitsch and Kempner, in speaking of segmentation, refer to the hanging together of the young by their sharpened ends and their gradual separation from each other; but they maintain that the young parasites may develop into adults before the radial arrangement is broken up, as is shown in their figure 27 (Rabinowitsch and Kempner, 1899, pl. 3, fig. 27.) We have not seen them develop into adults while so arranged. The rosettes seem to become dissolved while their members are still young. We should think that figure 27 represented an autöagglutination of adults rather than an advanced stage of a rosette, for the reason that the members are evidently adults instead of young forms.

THE DURATION OF THE INFECTION.

Rabinowitsch and Kempner found that artificially infected white rats harbor these parasites in general from four to six weeks. They

saw them disappear from the blood in some cases in from one to two weeks, sometimes earlier. Two rats retained their parasites three to four months.

Wasielewski and Senn found trypanosomes five and one-half months after injection in several instances and no animal lost his parasites before six weeks.

Jurgens (6) found that his animals kept their blood parasites as a rule one to two months, seldom later. In two cases numerous parasites were found after seven months. Only once did he find the infection to last shorter than one month.

We found the duration of the infection to be seven to fourteen days. The long periods of infection of three to six months, or even of six weeks, we have not seen. The trypanosomes never returned spontaneously to the rats after they had once become free of them. We were unable to find any wild rats infected spontaneously, but the duration of such an infection is, according to Rabinowitsch and Kempner, much longer than an artificial infection of white rats. They state that in an infection of the wild rats the parasite, so far as they have observed, did not disappear from the blood.

MOTION OF THE TRYPANOSOME.

If a thin film of fresh blood is watched beneath a cover slip on a slide the parasites are seen to be in striking activity, darting among the corpuscles and pushing them aside, but never entering the corpuscles or engulfing them. The motion is too violent to be closely followed by the eye, so in order to gain an accurate perception of the mechanism of the motion the film must be very thin, so that the trypanosome will be subjected to the pressure between the cover and slide, and consequently his motion becomes very slow.

We see that the parasite generally moves with the flagellum (anterior end) in front, but if he meets an obstruction he withdraws for only a short distance in the direction of his blunt (posterior) end, and then resumes in his original direction. The movement takes place by means of the undulating membrane and the flagellum. When in motion the body of the parasite rotates on its longitudinal axis, thus moving in a screw-like manner. This causes the undulating membrane to appear as if it were spirally arranged around the organism. The waves, starting in the flagellum and traveling along the undulating membrane, are plainly seen. Motion will persist in a cover glass preparation for several hours.

SYMPTOMS IN RATS.

Rabinowitsch and Kempner mention loss of appetite, slight loss of weight and debility, but say that none died from the infection. They regard the disease in white rats as mild compared with the same in wild rats.

Jurgens reports severe sickness and death.

Many of our rats died. Plain evidences of sickness coupled with heavy blood infection were almost sure to give a fatal result. We made no pathological study of the internal organs.

STAINING.

In the beginning we attempted to stain the parasites with the ordinary aniline dyes. We used eosin, methylene blue, hemalum, carbo-fuchsin, and carbo-thionin, but all were very disappointing. We then turned to the Romanowsky double stain of methylene blue and eosin, Goldhorn's polychrome methylene blue and Jenner's stain, all of which give most beautiful effects indeed; perhaps the best is Romanowsky's stain. Some modification of this stain is the one which many of the investigators have employed within the last few years for trypanosomes. In the hands of the various experimenters this stain seems to give somewhat different effects. The two points on which they all agree are that the nucleus takes a beautiful rose red and that the protoplasm takes a blue color. Rabinowitsch and Kempner state, and it is clearly shown in their figures, that in the hind part of the adult parasite there is an oval or round spot of a homogeneous structure which takes the stain uniformly and intensely red. In the front end of the trypanosome is a larger body with a net-like structure which stains rose red. In their hands the protoplasm of the body and the free outer border of the undulating membrane and the flagellum stained quite uniformly blue. The web of the membrane remained unstained.

Wasielewski and Senn state that the protoplasm is almost homogeneous, stains a light blue, and has a fine granular structure. In it are one to three clear, oval areas, generally in front of the centrosome, which can be considered as vacuoles. With the Romanowsky stain they succeeded in giving a red tint, not only to the centrosome and nucleus, but to the free border of the undulating membrane, to the web of the undulating membrane, to the flagellum, and to the entire border of the body of the parasite. They state, however, that the tint of the centrosome, flagellum, undulating membrane, and "periplast" was a bluish red.

Laveran and Mesnil picture the centrosome and body of the parasite as blue, and the nucleus, flagellum, and undulating membrane as red. With Romanowsky's stain we gave a blue color to the body of the trypanosome. The nucleus took a rose red, and the centrosome was deep red. The flagellum and border of the undulating membrane were red, and the web of the undulating membrane was faintly red.

Wright's modification of Romanowsky's stain.—Wright (7), in speaking of Romanowsky's stain, which came out in 1891 as a differential stain for the chromatin and cytoplasm of the malarial parasite, refers to the difficulties and uncertainties attending the preparation of the stain until it became finally modified by Leishman (8), whose

method Wright has further simplified. Wright, in his directions for preparing the stain, says to add 1 per cent methylene blue to a one-half per cent solution of sodium bicarbonate and steam the mixture in an Arnold steam sterilizer for one hour, which renders the methylene blue polychromatic. When cold he adds eosin until the color changes from blue to purple and a metallic scum forms on the surface and a black precipitate appears in suspension. The precipitate is collected on a filter, dried, and dissolved in methyl alcohol. Wright gives the following summary for using the stain:

1. Make films of blood, spread thinly, and allow them to dry in the air.
2. Cover the preparation with the alcoholic solution of the dye for one minute.
3. Add to the alcoholic solution of the dye on the preparation sufficient water, drop by drop, until the mixture becomes semitranslucent and a yellowish metallic scum forms on the surface. Allow this mixture to remain on the preparation for two or three minutes.
4. Wash in water, preferably in distilled water, until the film has a yellowish or pinkish tint in its thinner or better spread portions.
5. Dry between filter paper and mount in balsam.

Goldhorn's stain (9).—Dry the film and fix in pure methyl alcohol fifteen seconds, wash in running water, stain in 0.1 per cent aqueous solution of eosin for thirty seconds, wash in running water, stain one minute in Goldhorn's polychrome methylene blue, wash in water, dry in air, mount in balsam.

The polychrome methylene blue is made as follows:

1. Dissolve 2 grams of methylene blue and 4 grams of lithium carbonate in 300 c. c. of warm water.
2. Heat in porcelain dish in a boiling water bath fifteen minutes.
3. Pour into a glass-stoppered bottle and set aside for several days.
4. Render only slightly alkaline by adding 4 to 5 per cent acetic acid solution.

With this stain we have obtained beautiful preparations showing the chromatin of the ring form of æstivo-autumnal malaria and the chromatin of the tertian parasite. We have also well-stained preparations of blood platelets. It is a good stain for the nuclei of animal parasites. It shows the chromatin of the segmenting bodies of malaria, the chromatin of the crescents, the eosinophilic and neutrophilic granules and nuclei of leucocytes. Mast cells and myelocytes are well stained.

Jenner's stain.—This stain is not so good for trypanosomes as the other two. Equal parts of 1 per cent aqueous solutions of eosin and methylene blue are mixed and set aside for twenty-four hours. The mixture is filtered, the precipitate is washed with water and dried and then dissolved in methyl alcohol. In using this stain no previous fixing is necessary. After staining one to three minutes the specimen is thoroughly washed until the corpuscles appear pink. Dry in the air and mount in balsam.

The stain can be bought ready for use from dealers, or a powder

can be gotten from Grüber, which is to be dissolved in methyl alcohol.

We have gone at some length into these stains, for without some one of them the investigator will attain little in the way of getting instructive preparations of trypanosomes and because their more general use may bring out some points still unmentioned in the structure of animal parasites generally. Since a thorough understanding of the stains is necessary to fine work, the reader is referred to the original articles mentioned in the bibliography.

ACTIVE IMMUNITY.

With very few exceptions a single infection with trypanosomes renders the rats free from parasites thereafter. Rabinowitsch and Kempner had no second infections following the injection of heavily infected trypanosome blood into the peritoneal cavity of rats, which, after artificial inoculation, had become spontaneously free of parasites. Laveran and Mesnil, in their series of thirty, found two susceptible to a second infection. One of our rats infected by feeding and another infected by intrastomachal injection proved susceptible to a second infection by intraperitoneal inoculation in two and five months, respectively, after they had become free of parasites. Their second infections lasted only three days.

PASSIVE IMMUNITY.

From our knowledge of the antitoxins of the bacterial diseases, we are led naturally to the investigation of the protective property of the serum of immunized rats, and it is found that there is produced a specific immune serum. The serum of rats which have been immunized by one or more inoculations of trypanosome blood does give protection to other rats within certain limits. Laveran and Mesnil found that their most active serum came from a rat which had been given 13 inoculations.

If we add in vitro 1 c. c. of immune serum to 1 c. c. of trypanosome blood and inject the mixture into a fresh rat, no infection will follow. We also separated the two injections in time to see whether immune serum would prevent infection if injected before the trypanosome blood; likewise, whether the immune serum would prevent infection if it were injected after the injection of infected blood. Our results were somewhat variable, but in general they corresponded to the limits of time which have been set by Rabinowitsch and Kempner and confirmed by Laveran and Mesnil, namely, that 1 c. c. of immune serum injected into a fresh rat twenty-four hours before or twenty-four hours after the injection of trypanosomes will prevent infection. They found that emulsions of the spleen, bone marrow, liver, or brain conferred no passive immunity.

The limits of the preventive and curative power of the immune

serum, although quite narrow, are, however, as wide as we would expect.

Five pregnant rats were inoculated, with a view to finding whether their young would acquire an immunity by placental transmission. Although the mothers bore a very heavy infection, we could never demonstrate any parasites in the fetuses nor did the young show any evidences of an increased resistance to subsequent infection. Laveran and Mesnil mention one immune rat which had two litters; the first litter was immune, the second susceptible. In this connection we may mention that no case of placental transmission of malaria has been reported in which the possibility of postnatal infection has been excluded.

SUSCEPTIBILITY OF ANIMALS TO *TRYPANOSOMA LEWISI*.

The wild rat and the sewer rat are the only animals in which there is a spontaneous conveyance to each other. The white and spotted rats are susceptible by inoculation only. Young rats are more susceptible than old ones. Rabinowitsch and Kempner report the failure to infect pregnant rats. We inoculated five pregnant females, and all bore heavy infections. No one has found that the white rats harbored trypanosomes spontaneously in their blood. No investigators have succeeded in infecting other animals with rat trypanosomes except Laveran and Mesnil, who infected guinea pigs. We tried in vain to infect guinea pigs, rabbits, white mice, cats, a dog, a goat, and a horse by intraperitoneal inoculation. In one guinea pig we found six parasites in the blood twenty-four hours after inoculation, but subsequent examinations of the blood showed an absence of all parasites, so that this can not be considered a case of infection. Laveran and Mesnil, after intraperitoneal injection of a guinea pig with 1 c. c. of blood rich in trypanosomes, found multiplication forms in the peritoneum two to five days after injection. They had numerous failures in bringing about a blood infection, but some guinea pigs showed parasites in the blood in the proportions of 1:20 and 1:50 of the red blood corpuscles. The infection was of short duration.

When attempting to infect the various animals other than rats we injected large amounts of heavily infected rat blood. In some cases we injected a rat's entire blood. White rats and wild rats are so susceptible that only one to three drops of infected blood, mixed with a little sterile salt solution or bouillon and injected intraperitoneally, will cause a marked infection.

AUTO-AGGLUTINATION.

We have brought forward the use of this term to signify the agglutination of a rat's own trypanosomes while still circulating in his own blood. If daily examinations are made of the blood of an infected rat,

the parasites will be seen to show agglutination during the period of their decline in numbers and disappearance from the blood. When auto-agglutination is well advanced we see very few parasites occurring singly. They are collected into masses; but these masses in turn show a tendency to collect close together, which must necessarily leave certain drops of blood almost free from all parasites while other drops will show typical fields of auto-agglutination. On this account a single hanging drop or a single stained preparation taken from a rat is not sufficient to give a correct idea of the condition of the parasites in the blood. As many as eight to ten slides may be made in which will be found only a few scattered parasites, and the next slide will show pictures such as are seen in Plate II, figure 8, and Plate IV, figures 13 and 14. Figure 13 represents a single focus of agglutination, and in figure 14 there are three such foci near to each other.

A close examination of these agglutinations will show that they have nothing to do with the rosettes of multiplication. They are found in the blood after the period of multiplication has passed, and they are made up of adult parasites instead of young forms. We consider these agglutinations as an evidence of the presence of agglutinin in the rat's blood and as an omen of an impending rapid disappearance of the parasites from the blood. We have in the laboratory in several instances seen the agglutination of the parasites in a rat's blood for a few successive days before a sudden disappearance of all parasites over night. We conclude from this observation that agglutination is a step toward dissolution, and that it foretells the disappearance of the trypanosomes from the blood.

From a daily study of the blood of numerous cases in which agglutinations of parasites were present we were able to prophesy that active immunity was near at hand.

It is not every case of infection which shows auto-agglutination. We would explain its absence on the ground of an insufficient generation of agglutinin. As will be referred to later, there is a difference in the agglutinating power of the immune sera of different rats. As soon as a rat loses his parasites he is considered an immune and his serum is admitted to have agglutinating power on fresh parasites to which it may be added. The agglutinating power of the immune serum is, moreover, attributed to the agglutinin which it contains. Now, we think that the production of this agglutinin is a gradual process which is begun before a rat loses his parasites, and we think that just previous to the disappearance of all parasites from a rat's blood there is a short period during which he may generate sufficient agglutinin to agglutinate the parasites circulating in his own blood. We would offer as a possible explanation of our own cases that perhaps our trypanosomes were more virulent than those used by other observers. This view seems to be supported by the shorter time which elapsed between inoculation and heavy blood infection, the

more rapid disappearance of the parasites from the blood, and the greater number of deaths among the white rats.

The agglutinated parasites have a most orderly arrangement in the shape of a rosette, with their posterior ends close around a central point and their flagella at the periphery. Each parasite has its own fine undulating motion.

AGGLUTINATION.

1. *By immune serum.*—Immunity and agglutinating power go hand in hand. As in late years agglutination has been proven for so many kinds of bacteria by their specific sera, so trypanosomes are found to respond in a somewhat similar way to immune serum.

Laveran and Mesnil probably made the most complete study of agglutination. The trypanosome blood which they used was subjected to defibrination, which of course left the corpuscles and the trypanosomes in the serum. We have found that the substitution of clotting for defibrination will show the agglutination to much better advantage in the hanging drop, since there will be no corpuscles in the field to obscure agglutination. The immune blood and the trypanosome-bearing blood may be drawn up into separate fine capillaries and allowed to coagulate. The clot is then drawn out at one end of the tube, leaving the clear immune serum behind in the one case and the clear serum containing trypanosomes in the other case. Now the dilutions can be made just as in the Widal reaction. We have, however, taken another precaution which Laveran and Mesnil did not observe. We removed all agglutinin from the trypanosome serum before starting the tests. We were led to this by the study of auto-agglutination.

If we should select for our agglutination tests blood in which auto-agglutination was already noticeable, we would immediately fall into the error of getting agglutination with ever so great a dilution of the immune serum, because the trypanosomes were agglutinated before we began. Again, if we selected blood in which there was no auto-agglutination, but in which there was considerable agglutinin, but still not enough to produce an auto-agglutination, we would still be in error if we attempted to determine the agglutinating power of an immune serum by testing it on trypanosome-bearing serum which was just on the verge of auto-agglutination. We must therefore take into consideration one element which does not enter an agglutination test on bacteria. The bacterial pure culture has a fixed, uniform composition, and until we are able to grow trypanosomes in pure culture on artificial media we will have to consider the element of agglutinin in the serum which contains the parasites.

Our plan was to draw the trypanosome blood from the rat, allow it to coagulate, draw off the serum containing the parasites, and dilute it with plain distilled water and filter it through a porcelain filter.

under the influence of a vacuum. After four or five washings with large amounts of water, we let it filter until there remained behind a volume of fluid which equaled the original amount of serum. In this residue were the trypanosomes free from agglutinin. Dilutions were then made of the immune serum, and it was tested on the washed parasites. Some immune sera will not agglutinate in a dilution greater than 1:1. An agglutinating power of 1:5 or 1:10 is common. One of our rats showed typical agglutination in a dilution of 1:200.

It is interesting to watch in hanging drop an agglutination by a weak serum. At first two or three parasites are seen, joined by their posterior ends. Others come up toward the center of agglutination, recede for some distance, and later join the others. Some disengage themselves from the rosette and then rejoin it, until finally a well-arranged rosette is formed. Two small rosettes will gradually approach each other and then unite to form one mass, which in turn is joined by others of smaller or larger size.

A remarkable fact is that the agglutinated parasites do not lose their motility. There is not the diminution of motility before agglutination that is seen in a typhoid reaction, and while agglutinated each parasite retains a regular vibration. In agglutination with a strong serum the process takes place rapidly. The parasites rush together in great numbers, and the masses may be of macroscopic size. If a strong specific serum is used, the agglutinated masses are very compact and the individual parasites are tightly drawn together, so that there is little motion. In a general way the parasites are all pointed toward a center, but still they overlap and cross each other very much. With a weak serum the parasites are held together in a loose manner, permitting of more individual movement to each organism and more orderly arrangement, and there are fewer parasites to each rosette.

We often found agglutination almost complete, in which case very few parasites were to be seen free in the field. It may be only partial. The parasites may remain agglutinated until their death, or, if the serum is weak, a disagglutination may follow. Specific sera exposed to 55° C. for thirty minutes did not lose the agglutinating power, but a temperature of 65° C. maintained for half an hour destroyed its activity.

Laveran and Mesnil found that trypanosomes killed by chloroform or formalin were agglutinated by the same sera which agglutinate the living, but the parasites have no orderly arrangement in the mass. It is a remarkable fact that Rabinowitsch and Kempner found that "the trypanosome serum shows in no way whatever the property of agglutination."

2. *By normal sera.*—The action of normal sera in dilution of 1:1 was tested on the trypanosomes. The cat and horse sera were strongly agglutinating. The goat and rabbit sera were feebly agglu-

tinating. The sera of the white rat, white mouse, and guinea pig were negative.

Laveran and Mesnil state that the normal sera of the chicken and horse caused complete agglutination. The sera of the sheep, dog, and rabbit produced a partial reaction, while the pigeon, frog, guinea pig, white and spotted rats, and sewer rats caused none whatever. They also state that, although the blood of chicken and horse have agglutinating power, they do not protect against infection.

3. *In the ice chest.*—If trypanosome blood is drawn from the rat under aseptic conditions and sealed in pipettes and placed in the ice box, the parasites will usually join into beautiful rosettes after twenty-four hours. We found them agglutinated in blood which had been eighty-three days in the ice chest.

TRANSMISSION OF THE DISEASE.

1. *By intraperitoneal inoculation.*—Although white and spotted rats have never yet been found to harbor trypanosomes spontaneously in their blood, we find in them a very susceptible host for experimental inoculations, and it was with them that most of our work was done. Comparative studies show that a heavy blood infection is obtained by intraperitoneal injection sooner than by any other form of inoculation. The blood to be injected is mixed with 1 c. c. of saline solution or bouillon and injected with a hypodermic syringe. The period which elapses between the intraperitoneal inoculation and the first appearance of the parasites in the blood is variable, depending upon the amount of trypanosomes injected and the stage of development of the injected parasites.

Rabinowitsch and Kempner place the time at three to seven days, although they observed parasites in a few instances within the first day.

Laveran and Mesnil give three to seven days as the average time before the blood infection. They found a few in the circulation after five or six hours.

Jourgens found that the first presence of parasites in the tail blood occurred three or four days after inoculation, seldom later. After inoculation with 1 c. c. of blood he found them in tail blood in several instances after twenty minutes.

The parasites appeared in the tail blood in our cases usually on the second day. This was chiefly because we injected as a rule larger doses and used heavily infected blood in which were many developmental forms.

There has been considerable discussion as to where the principal seat of multiplication takes place in intraperitoneal inoculation. Rabinowitsch and Kempner regard the peritoneal fluid as a better nutritive medium for the development of the parasites than the blood and think that the chief seat of development and multiplication is in the peritoneum. In one to five days after intraperitoneal injection

of trypanosome blood they found in the peritoneum several engaged in development. They think that as soon as development is perfected in the peritoneum the parasite disappears from the peritoneal fluid into the blood. They give some weight to the fact that in one case of intravenous injection of parasites multiplication did not occur until the fifth day. Laveran and Mesnil state that there is less multiplication in the blood than in the peritoneum.

Our observations speak for the blood as the principal seat of development. On examination of the peritoneal fluid at varying times after injection we did not find division forms, nor did we find the first existence of parasites in the tail blood accompanied by division forms. The most reasonable explanation seems to be that if a large amount (0.75 c. c.) of heavily infected blood be injected into the peritoneal cavity, the small young forms and the long slender adults pass at once by the lymphatics into the general blood stream in sufficient numbers to be detected in cover-slip preparations within twenty-four hours or even within twenty-minutes, but the rosettes and other large division forms which are injected into the peritoneal cavity are prevented by their size from passage through the lymph channels and remain behind in the peritoneal cavity until their division is complete, when the young then pass through the lymph passages into the blood, leaving the peritoneum permanently free from parasites.

Daily examination of the blood for one to two days after the first appearance of parasites in the tail blood shows at most only a very gradual increase in the number of parasites present, but suddenly there comes an enormous swarming of the blood with parasites, and the presence of rosettes and other division forms indicate that multiplication is going on in the blood. The slide from which the microphotographs (Pl. III, figs. 11, 12) were made shows at least two dozen rosettes and was taken from the rat on the second day after the first appearance of parasites in the tail blood. It is not unusual to find parasites in the proportion of 1:2 red blood cells. Exceptional instances are met with in which the blood corpuscles are outnumbered by the trypanosomes. The duration of the period of multiplication is often no more than twelve to twenty-four hours. By the fourth day after the first entrance of parasites into the blood the height of infection has been reached. Rabinowitsch and Kempner found no division forms in the blood on the fourth day after the first appearance of parasites in the tail blood.

2. *By subcutaneous inoculation.*—Blood infection occurs by this form of injection a little later than by intraperitoneal inoculation. The shortest time in our cases between inoculation and the appearance of parasites in the blood was three days. Multiplication proceeded in the blood until it swarmed with myriads of parasites. This would seem to be additional evidence to the superiority of the blood over the peritoneal fluid as a nutritive medium for the development of the parasites, for in cases where the parasites had advantage of

the peritoneal fluid in addition to the blood their number did not reach a point beyond the number obtained by subcutaneous injection.

3. *By intrastomachal injection.*—We have read some discussion bearing on the natural mode of infection with trypanosomes in which infection followed the eating of a trypanosome rat by a healthy one. It was held that this could not be considered a case of intrastomachal infection because the possibility of the entrance of the parasites through wounds about the mouth, lips, or teeth had not been excluded.

We therefore arranged a series of experiments in which we thought all likelihood of infection through wounds was removed and that infection occurred through the stomach. Twelve white rats were chloroformed sufficiently to prevent any struggling. Then a small-sized catheter well oiled was passed into the stomach without encountering any resistance; injection of trypanosomes was made through the catheter, and the rats were then placed in separate cages and examined daily for parasites in the tail blood. Eleven out of the 12 developed blood infections fully as heavy as was obtained by any other form of inoculation. The time which elapsed before their appearance in the blood was as follows: Two in four days, one in five days, three in six days, three in seven days, and two in eight days.

We see that infection is considerably delayed by this method, the earliest being in four days and the latest in eight days.

4. *Transmission by feeding.*—The success of the intrastomachal injections naturally lead to a series of experiments to determine whether infection would not occur by feeding when all precautions were taken to prevent any wounding about the teeth or mouth.

Seven white rats, apparently free from mouth wounds, were put into separate cages to prevent fighting, and they were fed with soft food, so that no wounds would result from the gnawing of bones. They were each given a single feeding with the entire blood of a trypanosome rat. No other part of the infected rats was given to them. Trypanosomes appeared in the tail blood in five of the seven at periods of time which averaged six days.

Wild rats were then subjected to similar feeding experiments. In the blood of five wild rats we found parasites after three, seven, eight, nine, and ten days. Some of the rats had enormous numbers in their blood, while others had comparatively few.

We conclude from these experiments that infection may take place through the digestive tract and that the spread of the disease among wild rats may be due to feeding upon one another, especially since the instinct of fighting and pluck is so well implanted in them and is brought into action on slight provocation. We found it necessary to separate the wild rats in our stock cage to prevent losses from injuries inflicted on the weaker ones by the stronger.

After beginning the feeding experiments we were surprised to read the results obtained by Rabinowitsch and Kempner. They made intrastomachal injections through a stomach tube in four rats, but were

unsuccessful, although they repeatedly introduced into one rat blood rich in parasites and nourished one rat chiefly by this material. They placed together an infected tame rat and a noninfected wild rat, and state that after a hot combat the tame one was killed by the wild rat and eaten with much pleasure. Parasites appeared in his blood after ten days. They did not regard this as a case of infection necessarily by the digestive tract, as the parasites may have entered by the numerous bites and wounds, nor do we regard it as such; but we think we have in our experiments removed the likelihood of entrance through wounds and have established the existence of infection through the digestive tract alone.

5. *Transmission by fleas*.—Rabinowitsch and Kempner, with a view to finding the natural mode of transmission, placed together an infected white rat with a healthy white rat. The latter became infected in eleven days. They repeated the experiment and found parasites in the blood of another rat after fifteen days. A gray rat showed parasites in his blood after fifteen days' confinement with two infected animals. The three rats which became infected had many fleas on them. Examination of a great number of teased preparations of the fleas did not reveal any of the trypanosomes in them. They then mashed up fleas collected from infected rats and injected this material into the peritoneal cavity of nine white rats. Five became infected. Likewise four rats were injected intraperitoneally with mashed-up lice, but no infection followed. The next experiment was to determine whether the bites of fleas were infective. Twenty fleas were collected from infected rats and placed on one healthy white rat, which after three weeks time was found to be infected with trypanosomes. They say that from this one positive experiment they conclude that fleas can carry trypanosomes, and in the absence of proof of another way of conveyance they are of the opinion that fleas are the ordinary medium of infection.

Jourgens states that his experiments were not then complete, but that he had kept infected rats and healthy rats together in the same cage without infection taking place, although the animals were strongly beset with fleas, while a later inoculation of the sound animals proved them to be susceptible.

6. *Trypanosomes in lice*.—Laveran and Mesnil found trypanosomes in the stomachs of lice which infested infected rats, but do not report a conveyance of the disease by lice bites.

7. *Trypanosomes in Stegomyia fasciata*.—We may be pardoned for mentioning a subject which is entirely outside of rat trypanosomes—but it may have some future bearing on the disease—to state that within a year Durham (10) has reported finding trypanosomes in a mosquito, thus adding one more to the rapidly growing list of diseases through which this little creature threatens the public health. Durham's report is inserted.

A small bat (*Phyllostoma*) which could not be examined at once was placed in a gauze cage, and with it a specimen of *Stegomyia fasciata*. The next day the bat

was dead and the mosquito full of fresh blood. This blood contained abundant trypanosomes, whose shape is quite different from the usual ones found in rats, Nagana, etc. * * * Although flagellates, coccidia-like bodies, etc., often were found from time to time in the 80 mosquitoes which were dissected, this was the only time that trypanosomes were found.

THE PARASITES OUTSIDE THE BODY.

We must confirm the reports of Wasielewski and Senn, and of Rabinowitsch and Kempner, that if hanging drops are made of blood containing parasites in different stages of division a careful watching of the specimen will not witness the completion of the division process, whether the specimens be kept at room temperature or in the ice box or in the incubator.

Jurgens found quite the contrary. He made three hanging drops of the same blood with the same platinum loop. No. 1 was placed at 37° C. No. 2 was kept at room temperature. No. 3 was immediately dried, fixed, and stained, and examination showed it to contain parasites preparing to divide. On the next day Nos. 1 and 2 were dried, fixed, and stained. The stained specimen of No. 2 always showed the same conditions as No. 3; but in the stained specimen of No. 1 there were rosettes and division forms, none of which was seen in No. 3. Therefore, he concludes that it is possible for certain stages of the parasite under certain conditions to increase outside the body.

It will require further investigation to determine whether the parasite exists in the flea's body in the forms known to us as young forms, division forms, and adult parasites, or whether there is still another form which has not yet been described. The fact of not being able to find in infective fleas any form resembling a trypanosome has a close parallel in what occurred in one of our rats. A white rat in whose blood we saw many parasites in agglutination for several days suddenly showed an absence of parasites from the blood. We immediately performed an autopsy and made a careful search of the lungs, liver, spleen, kidneys, heart, brain, and bone marrow for parasites, but could find none. We were struck by the amount of granular débris in the liver and kidneys, but could not see any trypanosomes in them.

An emulsion was made of the kidneys and injected into the peritoneal cavity of a young rat. The liver was treated in the same manner and injected into two young rats. On the ninth day all three rats had parasites in their blood and a few days later developed heavy infections. While some unknown form of the parasite may have been present, it is of course possible that well-known forms of the trypanosomes were in the kidneys and liver and escaped notice.

Jurgens produced infection with 0.000005 c. c. of blood. If this amount of blood be added to 1 or 2 c. c. of salt solution, the dilution of the parasites would be so great that they might readily be missed in hanging-drop preparations.

The possibility of the existence of some very minute form of the

parasite was perhaps excluded by our filtration experiments. Trypanosome blood was diluted with fifteen parts of physiological salt solution and subjected to a porcelain filter under the influence of a vacuum. The filtrate was found to be noninfective to rats.

In blood kept in the ice chest for eighty-one days we found living trypanosomes. Their motion was of a trembling, vibrating character. Very few crossed the field. Many were arranged in rosettes. In the stained preparations we found parasites in which the positions of the centrosome and nucleus were reversed, the centrosome being anterior to the nucleus. In many of the trypanosomes there was granular degeneration, and there was also much free granular débris, which probably represented the remains of degenerated parasites. At room temperature and at 37° C. the parasites are short-lived. The different investigators found them capable of producing an infection of rats after being kept in the incubator and at room temperature from four to seven days. The parasites when kept at 12° to 16° C. in sterile pipettes can maintain their vitality very much longer than under any other condition.

Laveran and Mesnil kept parasites alive in defibrinated blood or in equal parts of defibrinated blood and physiological salt solution for forty-seven days in the ice box. At the end of this time the blood was injected into rats and produced a blood infection in nine days. In blood kept under the same conditions for fifty-one days they saw no parasites by microscopic examination, but it was infective for rats.

Jurgens found only a few parasites in blood kept in the ice chest for fifty-three days, but rats injected with it showed parasites in their tail blood after seven days.

Bacteria have a very detrimental effect on the life of the parasites outside the body. In hanging drops of trypanosome blood which has become infected with bacteria the parasites rapidly die. Blood drawn for keeping in the ice box must be kept under sterile conditions. Trypanosomes are killed after an exposure of a few minutes at 55° C. Wasielewski and Senn found living trypanosomes in the bloody urine of a rat. Rabinowitsch and Kempner were never able to find the parasites in the feces or urine.

THE TRYPANOSOME OF THE HAMSTER.

This animal harbors a parasite which has been studied by Rabinowitsch and Kempner. They find that it resembles the rat trypanosome in that it can hardly be differentiated from it morphologically and has the same process of development. On the other hand, they could not convey it to rats, and some of the rats which were refractory to hamster trypanosomes later proved susceptible to rat trypanosomes.

These facts, taken together with the fact that they could not convey rat trypanosomes to the hamster, led them to conclude that the hamster trypanosome and the rat parasite represent two different physiological varieties, which morphologically are almost inseparable.

TRYPANOSOMIASIS IN MAN.

Trypanosomiasis as a disease of man has not yet become acclimatized in the United States. At least no cases have been reported in this country. There have appeared in the foreign journals within the past year, however, eleven authentic instances of infection with trypanosomes.

A case of trypanosomiasis in a European, by Dutton (11) and Forde (12).—The patient was a European, 42 years of age, master of a government steamer on the Gambia River, in West Africa.

On May 10, 1901, the patient was admitted into the hospital at Bathurst, West Africa, suffering from what was regarded as malarial fever. Examination of his blood did not show malarial parasites, but there were seen extremely active bodies which were regarded as filaria. Three weeks later the patient was invalided to Liverpool, but returned in December, 1901, to Bathurst, where Dr. Dutton, of the Liverpool School of Tropical Medicine, examined the patient's blood and found the same parasite which Forde had probably seen seven months previously and which he at once recognized as a trypanosome.

The symptoms were an irregularly intermittent fever, a very marked erythema multiforme of the trunk and limbs, an œdematous condition of the face beneath the eyes and of the ankles, an acceleration of respiration and pulse rates, debility and loss of flesh, and enlarged spleen. The symptoms persisted throughout the eight months during which he was under observation and showed no reaction to treatment further than a slight abeyance under Fowler's solution.

Dutton found, while making examinations of fresh blood during the month of December, 1901, an average of one and one-half trypanosomes to each cover slip preparation. One preparation showed as many as 15 parasites. This case continued in its chronic course until the last week of life, during which week the disease assumed an acute type and the patient died January 1, 1903.

Dutton suggests the name *Trypanosoma gambiense* in case that further study shows it to be a new species.

A second case of trypanosomiasis in a European, by Manson (13).—The patient was the wife of a missionary on the Upper Kongo, where she had lived for a year. On account of sickness she returned to London.

Dr. Manson, of the London School of Tropical Medicine, recognizing the same group of symptoms which the patient of Dutton and Forde presented, made systematic, careful examinations of her blood daily for two weeks. During the two weeks no trypanosomes were found, but at the end of this time the parasites were readily seen in the peripheral circulation.

Trypanosomes in the blood of a West Africa native, by Dutton (14).—Three trypanosomes were found in a single smear from the

blood of a child 3 years of age, a native of Gambia. The child showed no symptoms of disease.

Eight additional cases of human trypanosomiasis.—In the British Medical Journal for February 7, 1903, there is a "preliminary account of the investigations of the Liverpool expedition to Senegambia," by Dutton, Annett, and Todd. They found trypanosomes in the blood of a white trader who had been twenty years in Gambia. The highest number found in a fresh preparation was seven. The patient had lost 45 pounds within the past year, complained of weakness and breathlessness, and had slight fever at times. The spleen was enlarged and there was pitting of the ankles. Four other cases of infection of natives were found.

Three more cases of human trypanosomiasis are reported in the British Medical Journal for March 28, 1903, by Dr. Patrick Manson. They came from the European community on the Kongo, as did also Dr. Manson's first case. A blotchy erythema and attacks of fever characterized these cases.

The finding of trypanosomes in man, associated with a well-defined group of signs and symptoms, is no small contribution to the disentanglement of the diseases of the Tropics. These cases will lead to the recognition of others, perhaps, in the tropical parts of our own continent or of Asia. The disease has been found in West Africa, and with this new fact in parasitology before us its geographical distribution may be found to have a much wider range. On account of the interest which surra claims in the Philippines and on account of the recognition within the past year of trypanosomes in man, and since the process of development and conveyance of these blood parasites have heretofore been little investigated in this country, we have undertaken this study.

We wish to call attention to the autoagglutination, transmission by feeding, transmission by intrastomachal injection, and to the staining.

I am glad to thank the director of the laboratory, Dr. M. J. Rosenau, for his interest in outlining the work.

I am indebted to Dr. John F. Anderson, the assistant director, for valuable suggestions, especially in the staining of the parasites.

I also desire to express my thanks to Dr. H. B. Parker for making the microphotographs.

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PLATE I.

Drawn with Abbe drawing camera. Stained according to Romanowsky method.
× 1,000.

Figs. 1 and 2. Adult trypanosomes.

Fig. 3. Parasite undergoing longitudinal division.

Fig. 4. Transverse division.

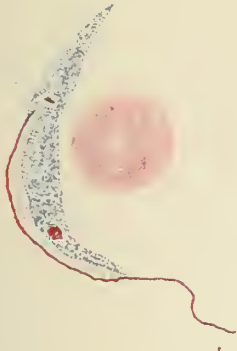


FIG. 1.



FIG. 2.



FIG. 3.

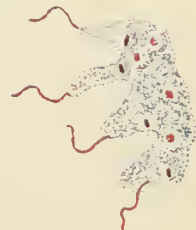


FIG. 4.



PLATE II.

Fig. 5. Beginning multiplication.

Fig. 6. Same, later stage. The mother parasite is still visible.

Fig. 7. A multiplication rosette.

Fig. 8. Auto-agglutination.

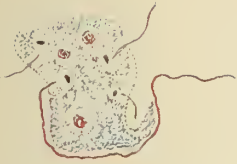


FIG. 5.



FIG. 6.

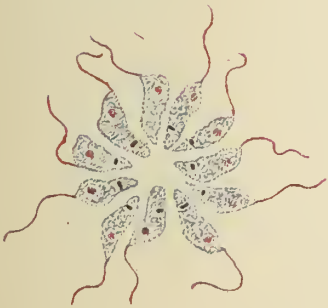


FIG. 7.

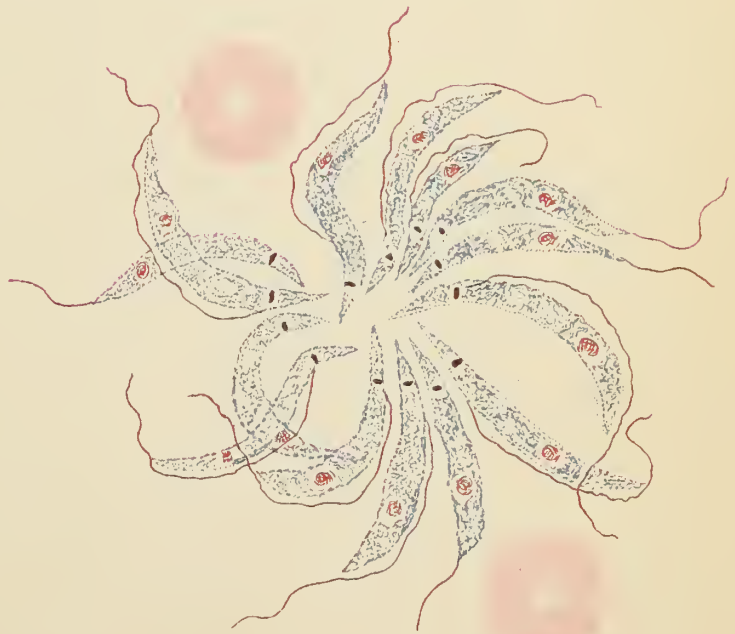


FIG. 8.



PLATE III.

Microphotographs. Stained with Goldhorn's stain.

- Fig. 9. Transverse division. The two new flagella coming off from the side which bears the undulating membrane.
- Fig. 10. Advanced stage of division. The parent parasite is curved around a red-blood corpuscle.
- Figs. 11 and 12. Multiplication by segmentation. The rosettes are composed of 9 and 14 daughter parasites.

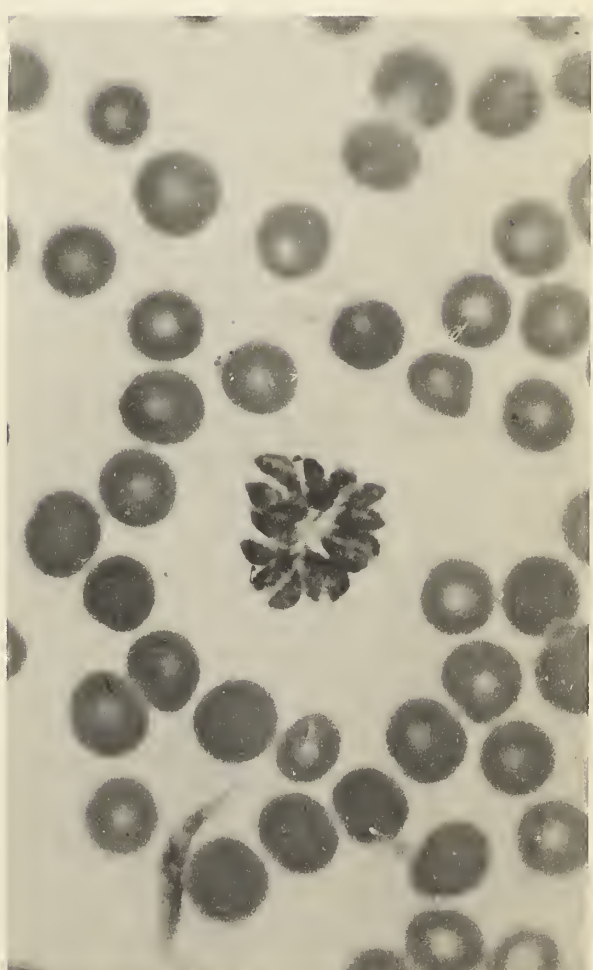
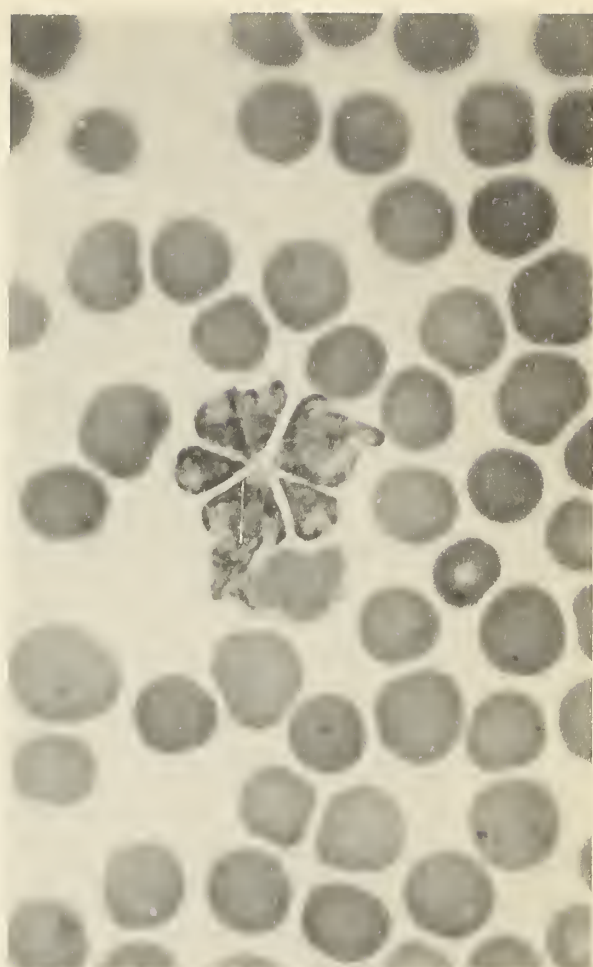
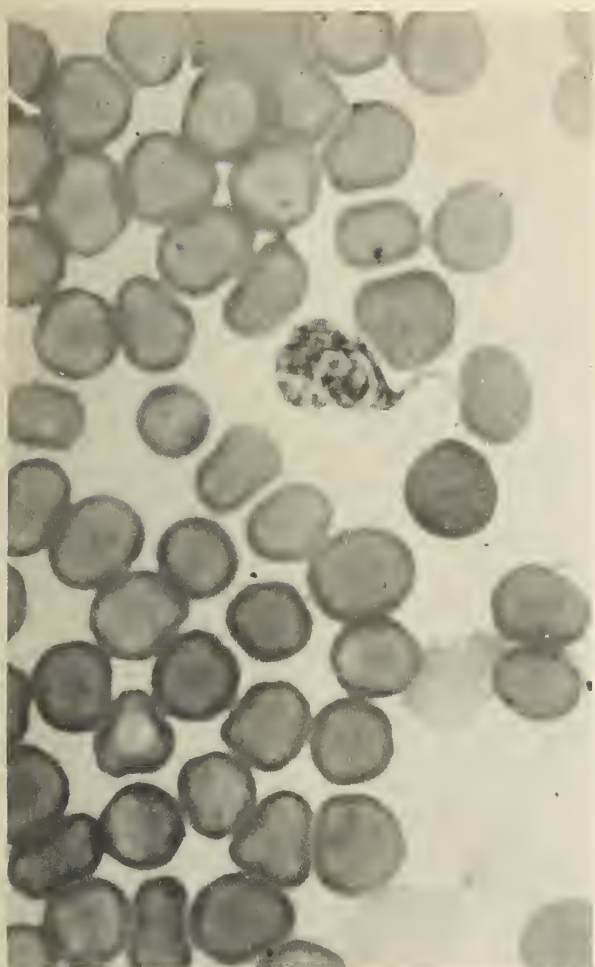
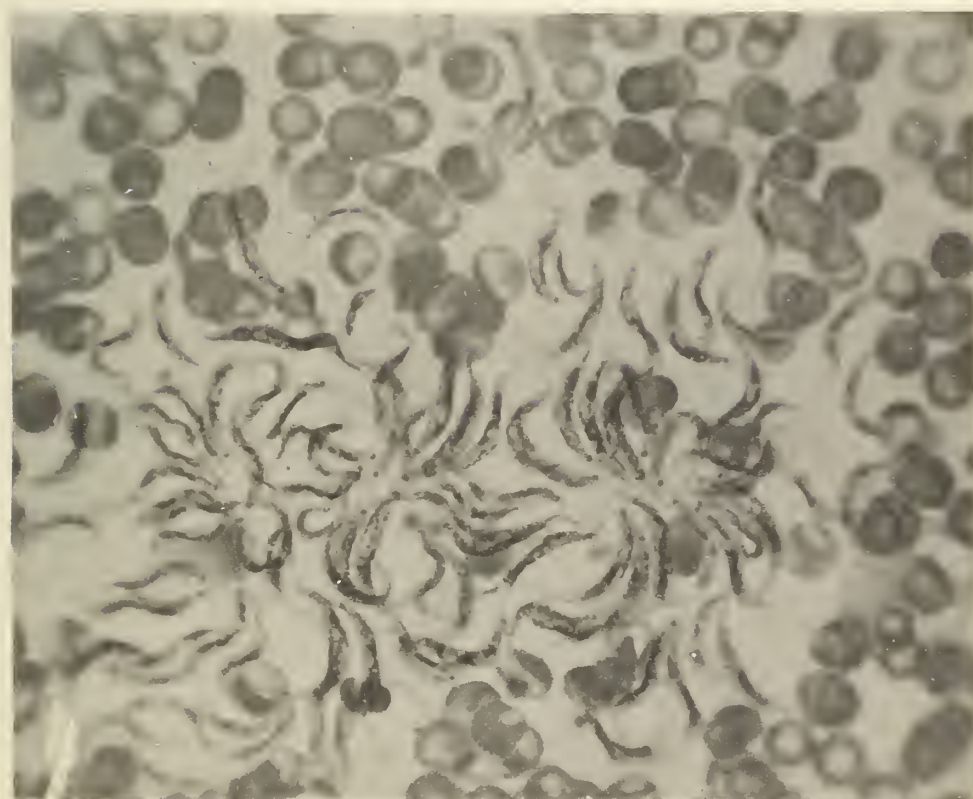
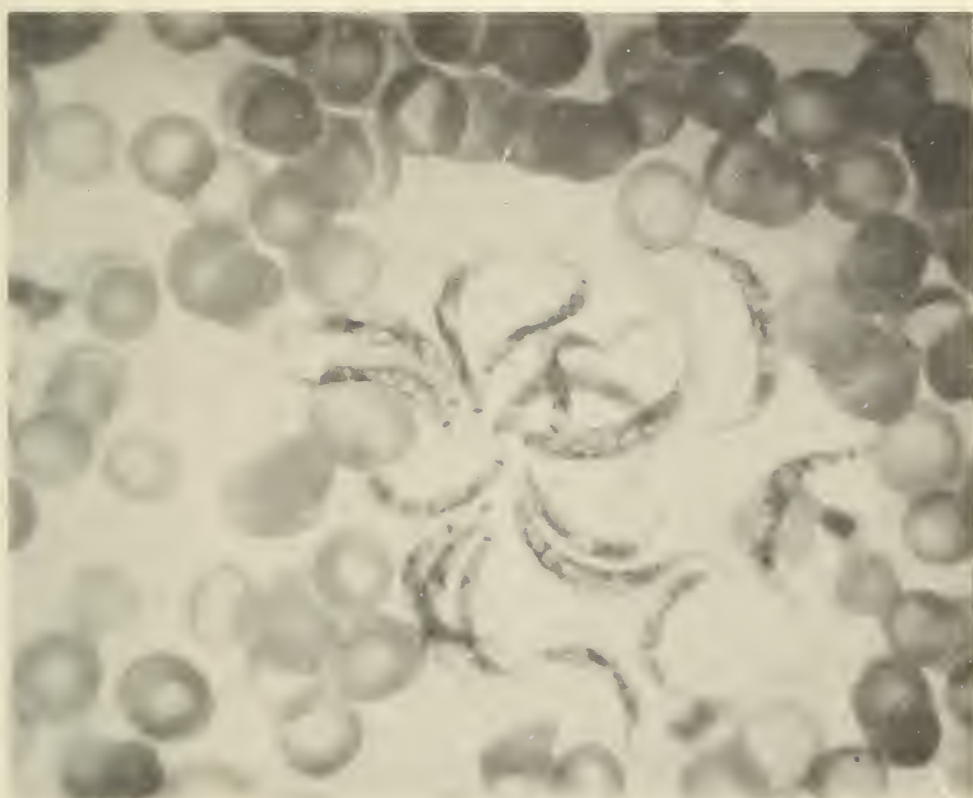


PLATE IV.

Auto-agglutination.

Fig. 13. Shows a single focus of agglutination.

Fig. 14. Shows three such foci.



TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

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THE BACTERIOLOGICAL IMPURITIES
OF
VACCINE VIRUS.

AN EXPERIMENTAL STUDY.

BY

M. J. ROSENAU,

DIRECTOR OF HYGIENIC LABORATORY.



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THE BACTERIOLOGICAL IMPURITIES OF VACCINE VIRUS. AN EXPERIMENTAL STUDY.

(By MILTON J. ROSENAU, Director of the Hygienic Laboratory, Passed Assistant Surgeon, U. S. Public Health and Marine-Hospital Service.)

OBJECT OF THE WORK.

The widespread prevalence of smallpox in our country during the past few years gave abundant opportunity to corroborate the great prophylactic value of vaccination. Hundreds of thousands of vaccinations have been performed during this period with very satisfactory and beneficial results. However, we were not surprised to hear of some accidents and complications. There were sore arms and a few fatalities resulting from wound complications.

The question immediately arose whether the cause of these complications was contained in the vaccinal matter or whether it resulted from subsequent contamination of the wound from outside sources.

Clinical studies can not be depended upon to settle this question, because infected "takes" may result from other causes than a contamination of the virus.

To determine this question, therefore, we made bacteriological studies of the number and kinds of micro-organisms that contaminate the different makes of vaccine virus found on the open market. In our studies special attention was given to the pus cocci and to tetanus.

We were rather surprised in visiting some of the vaccine farms to find that the operators permitted a certain degree of carelessness in preparing and collecting material for the glycerinated virus, trusting, as they stated, "to the glycerin to take out the impurities." This opinion of the exalted power of glycerin we have shown to be based upon error. Glycerin will destroy a certain number of bacterial impurities, but it is impotent against gross contamination or spores. Our studies of the germicidal power of glycerin will be made the subject of another paper.

All the vaccines examined were purchased in the open market from reliable pharmacists, who keep the virus under suitable conditions of light and temperature. Our results therefore represent the impurities found in the vaccine virus sold to the practitioner.

INEVITABLE IMPURITIES IN BOVINE VACCINE VIRUS.

Vaccinia is a specific disease, the cause of which has not been determined. We are, therefore, working somewhat in the dark. We are compelled to vaccinate our patients with a virus containing micro-organisms other than those causing vaccinia. The importance of using a virus as pure as possible need not be emphasized here, for we do not want to inoculate our patients with any other infection than the one which protects the individual against smallpox. On account of this danger human virus has been discarded in many countries, despite the fact that human virus is believed to be superior to bovine virus so far as the reliability and the duration of its immunizing power are concerned.

The production of bovine virus by propagating it from heifer to heifer is credited to Negri, of Naples, about 1842. It took some years for the advantages of this virus to be appreciated. Practically no other kind is now used in the large communities of Europe and in our own country. The great advantage of bovine virus, in addition to the ease with which it may be procured, is that it absolutely eliminates the possibility of the transmission of syphilis and other infections peculiar to the human family.

Now, although bovine virus is free from the danger of conveying the infectious diseases peculiar to man, it is liable to other equally undesirable contaminations. For instance, in addition to the micro-organisms that are specific for vaccinia, it contains the pus cocci and the bacteria that live normally upon and in the skin of the calf, and these micro-organisms always contaminate bovine virus. It is impossible to use germicidal agents in the treatment of the vaccinal eruption of calves, as such substances would kill the potency of the virus. Therefore, it is evident that even the greatest care will not insure such virus against "foreign" organisms.

OBJECT OF GLYCERINATING THE VACCINE PULP.

In order to eliminate the danger of the contaminating bacteria, Dr. Moncton Copeman, in 1891, devised the method of mixing the pulp with sterile glycerin of first quality. The advantages which Copeman claimed for the glycerin were that it not only prevented the growth and multiplication of the bacteria, always found in bovine virus, but gradually destroyed those which were present.

Glycerin can hardly be dignified with a place among the germicides, although the object of adding it to vaccine virus is to destroy the contaminating micro-organisms. Bacteria are slowly killed by glycerin, just as they are killed by drying; the glycerin is supposed to produce its bactericidal effects by a process of slow dehydration. Germs with thick envelopes resist it indefinitely. It has no action upon endogenous spores at all; in fact, it is a preservative of such infections as

tetanus, malignant edema, anthrax, and the like. In 80 and 90 per cent glycerin, we have kept as common and as easily destroyed an organism as the golden pus coccus alive forty-one days; the colon bacillus lived seventy days in 60 per cent glycerin; anthrax spores remained alive and virulent two hundred and seventy-eight days in glycerin. This work will soon be made the subject of another report.

Glycerin, therefore, can not take the place of care and cleanliness in the preparation of vaccine virus.

The effect of mixing glycerin with the virus is to gradually destroy both the bacteria and the vaccine, but fortunately the ordinary pus cocci and nonsporulating bacteria generally succumb before the viability of the vaccine organism is destroyed, and therefore there is an interval when the glycerinated virus will still cause a typical "take," but will contain comparatively few foreign micro-organisms. It is evident that if the glycerinated virus is used before this interval it has no advantage over the dry point, and if used after this interval it is inert. Therefore, from a theoretical view point glycerinated virus should be freer from impurities if used just at the right time. Manufacturers state that they usually glycerinate the virus from four to six weeks before putting it on the market.

The dry points, on the contrary, may be sold as soon as made and, if kept in a cool place protected from the light, they probably remain viable a longer time than the glycerinated virus under similar conditions. It is well known that pus cocci and the other bacteria which frequently contaminate vaccine virus die quickly when dry. These same bacteria, however, live a comparatively long time in dry vaccine virus, probably on account of the protection of the albuminous matter in which they are imbedded.

The glycerinated virus is made from the "pulp" that is scraped from the site of the eruption. This pulp consists of the inflammatory exudate and macerated cuticle. The mass is intimately ground with 50 to 60 per cent of glycerin, then placed aside in a cool place for several weeks until the pyogenic cocci and other pathogenic organisms disappear.

Until recently the dry points were always prepared by using the serum or "lymph" which exudes from the site of the eruption, after the crusts and epithelial layers have for the most part been removed. The sterile ivory points are then coated with this serum by means of a brush or the points are dipped directly into the exudate. This lymph is largely blood serum, mixed with inflammatory exudate. At present the dry points are mostly prepared from the glycerinated pulp. That is to say, the glycerinated virus, after it has stood a sufficient length of time to free itself of bacterial impurities, is dried upon ivory points. Sometimes the glycerinated virus is mixed with sterile blood serum in order to facilitate its drying and adhesion to the ivory point.

SEASONAL VARIATION IN THE PURITY OF VACCINE VIRUS.

A glance at the following tables will show the unevenness of the product of some manufactures. It will be noted that the contaminating microbes seemed to rise in numbers during the winter of 1901-2, when the demand for vaccine virus was very great, indicating perhaps haste in its production, or the marketing of an unripe product. It will also be noted that there is a marked improvement in the glycerinated product this winter (1902) as compared with that placed on the market last year. We believe that our warning, showing the feeble germicidal properties of glycerin, and an exposition of the fact that some of the glycerinated virus found upon the market was "green," helped materially toward this beneficial result. (See article in American Medicine, April 19, 1902, page 637, "Dry points versus glycerinated virus from a bacteriologic standpoint," read at a meeting of the New York Academy of Medicine, February 20, 1902, by M. J. Rosenau.)

Tables comparing the number of bacteria in the dry points and glycerinated virus marketed by the various manufacturers at different times.

MANUFACTURER NO. 1.

Dry points.			Glycerinated virus.		
Winter, 1901-2.	April and May, 1902.	November and December, 1902.	Winter, 1901-2.	April and May, 1902.	November and December, 1902.
4,923	7,240	0	192	22	1
6,240	7,326	0	1,332	24	2
9,050	7,625	1	1,456	28	2
9,289	7,625	1	6,249	46	2
9,884	9,430	2	6,876	72	3
10,629	10,050	3	7,274	101	3
14,826	10,340	3		48	4
20,828	10,345	3		80	4
	12,075	5		102	5
	12,100	11		110	9
				112	
				194	

MANUFACTURER NO. 3.

847	2,518	7,465	111	3,011	41
906	2,727	8,702	138	4,309	70
2,088	3,275	10,022	246	6,022	87
2,750	3,866	10,265	352	6,670	95
6,528	4,359	10,982	1,121	7,294	112
13,030	4,446	12,923	1,750	7,427	152
	5,577	15,552	2,070	9,309	230
	8,048	25,574	2,263	11,169	234
	8,384	26,411	2,440		239
	19,107	31,380			

Tables comparing the number of bacteria in the dry points, etc.—Continued.

MANUFACTURER NO. 4.

Dry points.			Glycerinated virus.		
Winter, 1901-2.	April and May, 1902.	November and Decem- ber, 1902.	Winter, 1901-2.	April and May, 1902.	November and Decem- ber, 1902.
1,530	636	243	1,414	24	3
2,160	1,998	262	1,540	54	4
2,376	2,069	420	1,842	68	6
8,024	2,433	424	2,928	84	11
9,688	2,726	452	10,372	97	16
12,800	2,780	465	11,232	144	19
	3,448	475	18,404	153	31
	3,960	479		165	37
	5,402	526		259	77
	8,160	576		393	113

MANUFACTURER NO. 5.

13	1	604	84	6	2
18	5	810	86	24	2
20	6	1,088	97	28	2
27	7	1,156	127	55	2
110	9	1,199	160	105	2
182	10	1,204	170		3
219	10	1,399	245		3
220	10	1,452	257		4
297	50	2,246	768		5
458		2,584	1,680		6
575			1,700		
648			2,069		
7,200			10,400		
11,200			17,000		
15,760					

MANUFACTURER NO. 6.

			369	21	0
			747	694	0
			1,592	1,877	0
			1,912	2,368	1
			2,106	2,689	2
			2,578	3,326	2
			3,819	4,208	4
				4,352	4
				8,094	5
					11
None examined.					

Tables comparing the number of bacteria in the dry points, etc.—Continued.

MANUFACTURER NO. 7.

Dry points.			Glycerinated virus.		
Winter 1901-2.	April and May, 1902.	November and December, 1902.	Winter 1901-2.	April and May, 1902.	November and December, 1902.
	63	3	116	28	4
	88	3	160	32	4
	95	5	1,600	35	5
	106	5	2,400	35	5
	120	6	8,000	37	6
	125	7		44	6
	128	21		47	7
	164	31		51	7
	342	48		59	9
	380	67		101	9

MANUFACTURER NO. 8.

450	2,548	853	2,100	13	8
476	3,405	868	2,200	14	10
516	3,805	887		20	11
3,325	3,829	1,800		21	12
3,475	4,026	1,847		21	13
3,600	4,321	1,946		21	14
	4,445	2,074		26	16
	4,665	2,244		28	16
	5,121	2,912		31	19
	5,355	3,754		42	20

MANUFACTURER NO. 9.

	195	5,952	11,840	12	7
	245	7,632	12,350	32	10
	312	7,560	12,960	116	13
	440	7,980	15,680	214	25
	496	8,748	16,480	236	43
	576	8,748	17,680	345	47
	578	9,744	17,920	370	70
	665	11,020	23,800	415	81
	805	12,972	28,640	416	85
	1,000	19,872	30,080	541	105

MANUFACTURER NO. 10.

			0	0	7
			0	0	14
			0	3	15
			0	3	17
			0	3	21
			0	4	22
			0	4	22
			0	5	31
			3	7	31
			4	9	53
None examined.					

TECHNIQUE EMPLOYED IN COUNTING BACTERIA IN VACCINE VIRUS.

The virus was suspended in a measured quantity of sterile bouillon and agitated so that all the clumps were broken up and a uniform suspension obtained as nearly as possible. The dry points were first softened in a small quantity of bouillon and then rubbed clean, always using the usual bacteriological precautions to prevent contamination from the outside. The glycerinated virus was mixed with a small quantity of bouillon, and the capillary tube washed out by drawing the liquid in and out of the tube a number of times.

The mixing was done in test glasses of appropriate size and the mixture thoroughly agitated. It is very important to agitate the suspension thoroughly in order to break up the clumps. This may best be done by drawing the fluid into pipettes and blowing it out vigorously, repeating the operation a number of times.

This suspension was now planted in agar and plated on Petri dishes. No less than three plates were made of each point or capillary tube; one or two drops of the suspension being planted in the first plate, five or ten drops in the second, and the total quantity remaining in the third plate. In this way the figures gave an accurate count of all the colonies that grew from each vaccine examined, excepting in those instances where the vaccine contained excessive numbers of bacteria. In such cases it was necessary to estimate the total number from the first or second plate.

The plates were grown in the incubator at 37° C., and the counts made when the maximum growth appeared, usually upon the third day.

It is practically impossible to determine the absolute number of organisms in a given virus, for it consists of an inflammatory product which is very variable in its physical characteristics. Upon dry points it coagulates into a hard, insoluble film; and mixed with glycerin it always contains little masses and agglutinated flakes and particles which hold enmeshed the micro-organisms. It is practically impossible to ultimately break up these masses. Therefore, the suspensions are not uniform and the counts we make are only approximations. Micro-organisms have a well-known tendency to group or cling together, so that every colony upon an agar plate does not represent the growth from one single microbe. The figures as given, therefore, are misleading only in that they give an underestimate of the number of organisms contaminating vaccine virus, and therefore some of the results, as bad as they are, do not fully represent the actual conditions.

The capillary tubes containing glycerinated virus vary considerably in capacity; some hold three and four times as much as others. This fact partly explains the discordance in some of the figures, but it is not sufficient to justify the marked discrepancy which we have found existing between tubes from the same package and bearing the same laboratory number as given by the manufacturer.

The following tables give the results of our counts, classified according to the manufacturer. They are given in three series: First, the result of our work during the winter of 1901-2; second, the result of our work during the summer (April and May); and, third, in November and December, 1902. We were surprised to find such a large number of colonies, especially from the glycerinated virus, and therefore repeated our work very carefully. The counts were made by Dr. Grubbs, Dr. Parker, Dr. Francis, and Dr. Anderson.

NUMBER OF BACTERIA IN VACCINE VIRUS BOUGHT ON THE OPEN MARKET, WITH A SPECIAL STUDY OF THE PYOGENIC COCCI.

The number of bacteria found in vaccine virus is of course not so important a factor as the kind of organisms. A few tetanus spores or virulent pus cocci would be of far more serious moment than myriads of the common saprophytes. However, the number of organisms found to contaminate vaccine virus is a fair index of its purity, as well as of the care taken in its production. The number of bacteria found in glycerinated virus is also an indication of its "ripeness."

We counted 190 dry points and 244 glycerinated tubes. The dry points averaged 4,354 bacteria per point and the tubes 1,742 per tube.

Some points contained as great a number as 44,000 foreign organisms. The highest count found in the capillary tubes containing glycerinated virus was 30,080. A few (very few) points and tubes were sterile, i. e., gave no bacterial growth either on agar plates or in bouillon.

SERIES 1.

The following tables give the results of our studies during the winter of 1901-2:

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
<i>a</i> 9, 289	<i>b</i> 192
<i>a</i> 14, 826	<i>b</i> 1, 332
<i>a</i> 20, 828	<i>b</i> 1, 456
<i>c</i> ———	<i>b</i> 6, 249
6, 240	<i>b</i> 6, 876
9, 884	<i>b</i> 7, 274
<i>c</i> ———	
4, 923	
9, 050	
10, 629	

a Orange, yellow, and white staphylococci were isolated from these points; not pathogenic for mice, rats, and guinea pigs.

b Yellow and white staphylococci were isolated from these tubes. They produced no effect upon mice, rats, and guinea pigs.

c These short lines separate samples bearing a different number as given by the manufacturer.

This virus evidently contains more bacteria than a good virus should. The ordinary cocci of suppuration were found in both the dry points and the glycerinated virus; and although these organisms when inoculated into laboratory animals gave no results, there is little indication that under favorable circumstances they might not be pathogenic for man. While the glycerin has reduced the average number of bacteria found in this virus, it still has pathogenic bacteria that it should not contain; and, so far as the numbers are concerned, the samples examined, with one exception (192), are far above the number allowed a good virus.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
847	2, 263
906	1, 750
2, 088	2, 070
2, 750	2, 440
6, 528	111
13, 030	138
	246
	352
	1, 121

A great many molds common to the air and to hay, were found in this virus, indicating stable contamination.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
1,530	10,372
2,376	11,232
<u>2,160</u>	<u>18,404</u>
8,024	1,414
9,688	1,540
12,800	1,842
	2,928

Here we have a manufacturer who puts up both kinds of virus, but his glycerinated product contains more bacteria than his dry points.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
110	84
182	768
220	1,680
575	1,700
7,200	2,069
11,200	10,400
15,760	17,000
<i>a</i> 219	<i>a</i> 86
<i>a</i> 297	<i>a</i> 97
<i>a</i> 458	<i>a</i> 160
<i>a</i> 648	<i>a</i> 257
<u> </u>	<u> </u>
<i>b</i> 13	<i>b</i> 127
<i>b</i> 18	<i>b</i> 170
<i>b</i> 20	<i>b</i> 245
<i>b</i> 27	

a Taken from the same lot and examined three months later.

b Another sample.

The only comment that it seems necessary to make on the product of this manufacturer is its unevenness. While the average is fair, we found two capillary tubes of glycerinated virus to contain an excessive contamination. It may be that these tubes were placed upon the market before they were sufficiently glycerinated.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	369
	747
	2,106
	1,592
	1,912
	2,578
	3,819

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	116
	160
	1,600
	2,400
	8,000

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
450	^a 2,100
476	^a 2,200
516	
^b	
3,325	
3,475	
3,600	

^aThe ordinary pus cocci (white, yellow, and orange) were isolated from this virus, but when inoculated into animals gave negative result.

^bThis line separates samples bearing a different number as given by the manufacturer.

Nine weeks later other samples of the glycerinated virus out of the same box were counted and gave only 30 bacteria per tube. This would seem to indicate that the virus was "green" when placed upon the market, and that if the manufacturer had kept it a few weeks longer it would have been freer from contamination.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	11,840
	12,350
	12,960
	15,680
	16,480
	17,680
	17,920
	23,800
	28,640
	30,080

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	0
	0
	0
	0
	0
	0
	0
	0
	3
	4

SUMMARY OF SERIES I.

Winter 1901-2.

Number of organisms found.		
Dry points.		Glycerinated tubes.
13	0	2,100
18	0	2,106
20	0	2,200
27	0	2,263
110	0	2,400
182	0	2,440
219	0	2,578
220	0	2,928
297	3	3,819
450	4	6,249
458	30	6,876
476	84	7,249
516	86	8,000
575	97	10,372
648	111	10,400
847	116	11,232
906	127	11,840
1,530	138	12,350
2,088	160	12,960
2,160	160	15,680
2,376	170	16,480
2,750	192	17,000
3,325	245	17,680
3,475	246	17,920
3,600	257	18,404
4,923	352	23,800
6,240	369	28,640
6,528	747	30,080
7,200	768	
8,024	1,121	
9,050	1,332	
9,289	1,414	
9,688	1,456	
9,884	1,540	
10,629	1,592	
11,200	1,600	
12,800	1,680	
13,030	1,700	
14,826	1,750	
15,760	1,842	
20,828	1,912	
	2,069	
	2,070	
Number points examined 41	Number tubes examined 71	
Total bacteria 197,185	Total bacteria 333,586	
Average per point 4,809	Average per tube 4,698	



SERIES II.

The following tables give the results of our studies during the spring of 1902:

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
7,240	22
7,326	24
7,625	28
7,625	46
9,430	72
10,050	101
10,340	—
10,345	48
12,075	80
12,100	102
	110
	112
	194

The dry points were dated “to be exchanged after June 3, 1902,” and were examined May 23, 1902. The plates contained many colonies of a yellow staphylococcus resembling pus organisms.

The glycerinated virus was dated by the maker “to be exchanged after April 25, 1902,” and was examined April 26, 1902.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
2,518	3,011
2,727	4,309
3,275	6,022
3,866	6,670
4,359	7,294
4,446	7,427
5,577	9,309
8,048	11,169
8,384	
19,107	

The dry points were guaranteed by the manufacturer until June 7, 1902, and were examined April 30, 1902.

The glycerinated virus was guaranteed by the manufacturer until June 17, 1902, and was counted April 30, 1902.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
636	24
1,998	54
2,069	68
2,433	84
2,726	97
2,780	144
3,448	153
3,960	165
5,402	259
8,160	393

The dry points were labeled by the maker to be returned after May 28, 1902, and were counted May 14, 1902.

The glycerinated virus was labeled by the maker to be returned after June 17, 1902, and was examined May 15, 1902.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
1	6
5	24
6	28
7	55
9	105
10	
10	
10	
50	

The dry points were labeled by the maker to be returned after May 18, 1902, and were planted April 25, 1902.

The glycerinated virus was labeled to be returned after May 11, 1902, and was examined April 25, 1902.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	21
	694
	1, 877
	2, 368
	2, 689
	3, 326
	4, 208
	4, 352
	8, 094

The glycerinated virus was stated by the manufacturer to be good until June 4, 1902, and was examined April 30, 1902.

Many yellow and white colonies grew on the plates from this vaccine resembling pus cocci. The following table shows the result of our study of 12 of these organisms:

Table of staphylococci isolated from

No.	Grouping.	Relation to Grams.	Indol.	Growth in milk.
³ 10	Staphylococci	Stains	Negative	Very slight acid product.
³ 10	...do	...do	...do	No change.....
⁴ 5	...do	...do	...do	Light yellow
⁴ 10	...do	...do	...do	Light brown.....
⁴ 0	...do	...do	...do	Light yellow
⁵ 10	...do	Negative	...do	Acid production; precipitate of casein.
⁶ 10	...do	Stains	...do	No change.....
⁷ 23	...do	...do	...do	...do
⁸ 5	...do	...do	...do	...do
⁹ 20	...do	...do	Very slight	Alkaline, brownish yellow precipitate.
¹⁰ 65	...do	...do	Negative	No change.....
¹⁰ 65	...do	...do	...do	Dirty light blue, with scum on surface.

glycerinated virus of manufacturer No. 6.

Growth in gelatin.	Growth in bouillon.	Growth on potato.	Growth in glucose bouillon.	Growth in lactose bouillon.
No liquefaction...	Diffused cloudiness.	White granular growth; scanty.	No fermentation; cloudy all through.	No fermentation; cloudy at bend of tube.
Slow liquefaction.	Slightly cloudy...	Heavy growth; yellowish green.	No fermentation; slightly cloudy at bend of tube.	Do.
No liquefaction...	do	Slight yellow growth.	No fermentation; cloudy at bend of tube.	Do.
.....do	do	No growth.....	No fermentation; very slight growth at bend of tube.	No fermentation; very slight growth at bend of tube.
.....do	do	Heavy yellow growth all over potato.	No fermentation; precipitate at bend of tube.	No fermentation; precipitate at bend of tube.
.....do	Cloudy	Heavy white growth spreading.	No fermentation; slightly cloudy at bend of tube.	No fermentation; slightly cloudy at bend of tube.
Very slow liquefaction.	do	Yellowish white growth; very heavy.	No fermentation; very slight growth at bend of tube.	No fermentation; very slight growth at bend of tube.
No liquefaction...	do	Invisible growth.	do	Do.
.....do	do	do	do	Do.
.....do	do	Abundant white growth, slowly spreading.	do	Do.
Slow liquefaction.	Slightly cloudy...	Slow growth, yellow granular.	do	Do.
No liquefaction...	Slightly cloudy; some precipitate.	No growth.....	do	Do.

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
63	28
88	32
95	35
106	35
120	37
125	44
128	47
164	51
342	59
380	101

The dry points were stated by the maker to be good until June 15, 1902, and were examined April 26, 1902.

The glycerinated virus was labeled by the manufacturer to be good until May 1, 1902, and was counted April 26, 1902.

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
2,548	13
3,405	14
3,805	20
3,829	21
4,026	21
4,321	21
4,445	26
4,665	28
5,121	31
5,355	42

Counted May 15, 1902. The packages contained neither date nor laboratory number.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
195	12
245	32
312	116
440	214
496	236
576	345
578	370
665	415
805	416
1,000	541

The dry points were prepared, according to the maker's label, May 1, 1902, and were counted May 15, 1902.

The glycerinated virus was not to be used after July 1, 1902, according to the maker's statement, and was examined May 15, 1902.

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined	0
	0
	3
	3
	3
	4
	4
	5
	7
	9

The glycerinated virus was marked to be returned after August 1, 1902, and was counted May 16, 1902.

SUMMARY OF SERIES II.

April and May, 1902.

Number of organisms found.			
Dry points.		Glycerinated tubes.	
1	5,355	0	110
5	5,402	0	112
6	5,577	3	116
7	7,240	3	144
9	7,326	3	153
10	7,625	4	165
10	7,625	4	194
10	8,048	5	214
50	8,160	6	236
63	8,384	7	259
88	9,430	9	345
95	10,050	12	370
106	10,340	13	393
120	10,345	14	415
125	12,075	20	416
128	12,100	21	541
164	19,107	21	694
195		21	1,877
245		21	2,368
312		22	2,689
342		24	3,011
380		24	3,326
440		24	4,208
496		26	4,309
576		28	4,352
578		28	6,022
636		28	6,670
665		28	7,294
805		31	7,427
1,000		32	8,094
1,998		32	9,309
2,069		35	11,169
2,433		35	
2,518		37	
2,548		42	
2,726		44	
2,727		46	
2,780		47	
3,275		48	
3,405		51	
3,448		54	
3,805		55	
3,829		59	
3,866		68	
3,960		72	
4,026		80	
4,321		84	
4,359		97	
4,445		101	
4,446		101	
4,665		102	
5,121		105	
Number points examined.. 69		Number tubes examined.... 84	
Total bacteria..... 238,626		Total bacteria..... 88,879	
Average per point..... 3,458		Average per tube 1,058	

SERIES III.

The following tables give the results of our studies made during the winter, November and December, 1902.

Note especially how vastly superior the glycerinated virus in this series is to Series I and II. Eighty-nine capillary tubes examined averaged only 29 bacteria per tube, whereas in Series II, made in April and May, 1902, the average was 1,058, and in Series I, made in the winter 1901-2, it was 4,698.

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
0	1
0	2
1	2 (Coc. 3.)
1	2
2	3
3 (Coc. 1.)	3
3	4
3	4
5	5
11 (Coc. 2.)	9 (Coc. 4.)

These points were labeled by the manufacturer to be exchanged after November 23, 1902, and were counted November 21, 1902.

The glycerinated lymph was labeled good to December 10, 1902, and was counted November 24, 1902.

The following organisms, some of which resemble the pyogenic cocci, were isolated:

Coccus No. 1.—This staphylococcus was one of 3 colonies which grew on our agar plates planted from a dry point of manufacturer No. 1.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon is slightly clouded with some white precipitate; on agar, a grayish white moist glistening growth; on potato, white growth, slowly spreading; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 2.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a dry point of manufacturer No. 1.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon is clear, with a yellow precipitate; on agar a lemon yellow, moist, shiny growth;

on potato, a scant yellowish growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 3.—This staphylococcus was one of 2 colonies which grew on our agar plates planted from a tube of glycerinated virus of manufacturer No. 1.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, a slight alkaline production; does not liquefy gelatine; bouillon is clear, some white precipitate; on agar, a heavy, glistening pink pigment growth; on potato, very scanty pink growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 4.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of glycerinated virus of manufacturer No. 1.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, no change, but a white precipitate; does not liquefy gelatine; in bouillon, clear, white precipitate; on agar, a scanty white, glistening growth; on potato, very scanty white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
7,465	41
8,702 (Coc. 5.)	70
10,022	87
10,265	95 (Coc. 9.)
10,982	112 (Coc. 10.)
12,923 (Coc. 6.)	152
15,552	230
25,574	234
26,411 (Coc. 7.)	239
31,380 (Coc. 8.)	

The dry points were labeled to be good until December 7, 1902, and were planted for study November 8, 1902.

The glycerinated lymph was labeled good until December 17, 1902, and was planted November 25, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 5.—This staphylococcus was one of 8,702 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, slightly acid production; does not liquefy gelatine; bouillon very cloudy, white pellicle, and precipitation; on agar, grayish-white, shiny, moist growth; on potato, heavy yellowish-white growth all over potato; in glucose bouillon, slight fermentation, cloudy all through; in lactose bouillon, slight fermentation, cloudy all through.

Coccus No. 6.—This staphylococcus was one of 12,923 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid produced in litmus milk; liquefies gelatine very slowly; bouillon is slightly cloudy, with some white precipitation; on agar, a grayish-white, shiny, moist growth;

on potato, a white, granular, spreading growth; in glucose bouillon, no fermentation, slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

Coccus No. 7.—This staphylococcus was one of 26,411 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; rapidly liquefies gelatine; bouillon is clouded with white precipitation; on agar there is a white, moist, shiny growth; on potato, a white, granular growth, nearly invisible; in glucose bouillon, no fermentation, but growth at bend of tube; in lactose bouillon, no fermentation, but growth at bend of tube.

Coccus No. 8.—This staphylococcus was one of 31,380 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; liquefies gelatine very slowly; bouillon is slightly cloudy, with white precipitation; on agar, a yellowish-white, moist, shiny growth; on potato, white, granular growth nearly invisible; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 9.—This staphylococcus was one of 95 colonies which grew on our agar plates planted from a tube of manufacturer No. 3. The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; does not liquefy gelatine; bouillon is very cloudy, white precipitate; on agar, a grayish-white, moist, shiny, glistening growth; on potato, very heavy, white growth all over; in glucose bouillon, fermentation 1½ inch, growth all through; in lactose bouillon, fermentation three-fourths inch, growth all through.

Coccus No. 10.—This staphylococcus was one of 112 colonies which grew on our agar plates planted from a tube of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; in bouillon, clear yellowish pellicle and precipitate; on agar, yellow, dry, and granular growth; on potato, very scanty, yellow growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
243	3
262	4
420	6
424	11 (Coc. 13, 14.)
452	16
465	19
475 (Coc. 11, 12.)	31
479	37
526	77 (Coc. 15.)
576	118

The label of this manufacturer stated that the dry points were good till November 28, 1902. They were planted for counting November 18, 1902.

The glycerinated virus was labeled to be good till January 4, 1903, and was planted for study November 28, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 11.—This staphylococcus was one of 475 colonies which grew on our agar plates planted from a dry point of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; rapidly liquifies gelatine; bouillon is cloudy, with some precipitate; on agar there is a whitish-yellow, moist, shiny growth; on potato, a yellow growth, spreading slowly; in glucose bouillon, no fermentation, but cloudy all through; in lactose bouillon, no fermentation, cloudy all through.

Coccus No. 12.—This staphylococcus was one of 475 colonies which grew on our agar plates planted from a dry point of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, acid production, coagulation and casein precipitate; bouillon is slightly cloudy, white precipitate; on agar, whitish gray, moist, glistening growth; on potato, white granular growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

Coccus No. 13.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly clouded, yellow precipitate; on agar, growth transparent, thin layer, no pigmentation; on potato, scanty yellow growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 14.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly cloudy, some white precipitate; on agar, a lemon-colored, moist, glistening growth; on potato, no growth; in glucose bouillon, no fermentation, but growth all through; in lactose bouillon, no fermentation, but growth all through.

Coccus No. 15.—This staphylococcus was one of 77 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly cloudy, with some precipitate; on agar, a slight granular, scanty growth, no pigmentation; on potato, no growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
604	2
810	2
1,088	2
1,156	2
1,199	2
1,204	3 (Coc. 16.)
1,399	3
1,452	4
2,246	5
2,584	6

These dry points were stated by the manufacturer to be good until December 4, 1902. They were planted for counting November 7, 1902.

The glycerinated lymph was stated by the manufacturer to be good until December 19, 1902, and was counted November 24, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 16.—This staphylococcus was one of 3 colonies which grew on our agar plates planted from a tube of manufacturer No. 5.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly clouded, with some white precipitate; on agar, a whitish gray, moist, shiny growth; on potato, a white spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
	0
	0
	0
	1
	2
	2
	4
	4
	5
	11 (Coc. 17.)

The glycerinated virus was dated August 8, 1902, and was planted December 3, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 17.—This staphylococcus was one of 11 colonies which grew on our agar plates, planted from a tube of manufacturer No. 6.

The staphylococcus stains by Gram's method. Does not produce indol in a 24-hour bouillon culture; produces acid, with yellow pellicle and precipitate in litmus milk; liquefies gelatine very slowly; bouillon clear, with some yellow precipitate; on agar, a heavy yellow-orange growth; on potato, scant yellowish growth; in glucose bouillon, no fermentation, slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
3	4 (Coc. 19.)
3	4
5	5
5	5
6	6
7	6
21	7
31	7
48	9 (Coc. 20.)
67 (Coc. 18.)	9 (Coc. 21.)

The points were labeled to be good till December 15, 1902, and were planted November 24, 1902.

The glycerinated virus was labeled good till December 15, 1902, and was counted November 22, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 18.—This staphylococcus was one of 67 colonies which grew on our agar plates planted from a dry point of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon cloudy with white precipitate; on agar, whitish transparent, slightly granular, scanty growth; on potato, brownish white granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 19.—This staphylococcus was one of 4 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; liquefies gelatine; bouillon cloudy, white pellicle and precipitate; on agar, white shiny growth; on potato, white granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 20.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon clear with slightly white precipitate; on agar, whitish gray isolated colonies; on potato, no growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 21.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; a slight acid production in litmus milk; slowly liquefies gelatine; bouillon cloudy with some white precipitation; on agar, heavy yellow growth; on potato, very heavy yellow granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
553 (Coc. 22.)	8
868	10
887 (Coc. 23.)	11
1,800	12
1,847	13
1,946	14
2,074	16 (Coc. 25.)
2,244	16
2,912	19
3,754 (Coc. 24.)	20

The dry points of this manufacturer have no date. They were planted December 5, 1902.

The glycerinated virus also had no date and was counted December 2, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 22.—This staphylococcus was one of 553 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon cloudy, with white precipitate; on agar, white, shiny, moist, glistening growth; on potato, yellowish white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 23.—This staphylococcus was one of 887 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; very slight acid production in litmus milk; liquefies gelatine very slowly; bouillon cloudy, with white precipitate; on agar, dirty white, shiny, moist growth; on potato, yellow spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 24.—This staphylococcus was one of 3,754 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid production in litmus milk; liquefies gelatine; bouillon is cloudy, with white precipitate; on agar there is a white, shiny, moist, glistening growth; on potato, a white granular growth spreading all over the potato; in glucose agar, no fermentation, but growth at bend of tube; in lactose bouillon, no fermentation, but growth at bend of tube.

Coccus No. 25.—This staphylococcus was one of 16 colonies which grew on our agar plates planted from a tube of manufacturer No. 8.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; very strong acid production in milk; does not liquefy gelatine; bouillon slightly cloudy, with some white precipitate; on agar, a slight granular scanty growth with no pigmentation; on potato, no growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth at bend of the tube.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
5,952	7
7,632	10
7,560	13
7,980	25
8,748 (Coc. 26.)	43 (Coc. 29.)
8,748 (Coc. 27.)	47
9,744	70
11,020	81 (Coc. 30.)
12,972 (Coc. 28.)	85
19,872	105

According to the label, the dry points were prepared by the manufacturer October 31, 1902, and were planted November 29, 1902.

The glycerinated virus was not dated. It was counted December 1, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 26.—This staphylococcus was one of 8,748 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; is slightly acid in litmus milk; does not liquefy gelatine: bouillon cloudy, with yellow precipitate; on agar, an orange moist glistening growth; on potato, heavy yellow growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

Coccus No. 27.—This staphylococcus was one of 8,748 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid production in litmus milk; does not liquefy gelatine; in bouillon, heavy white pellicle, with precipitate; on agar, an orange-yellow moist glistening growth; on potato, a yellowish granular growth; no fermentation, cloudy all through, in glucose bouillon; and in lactose bouillon there is no fermentation, but the bouillon is cloudy all through.

Coccus No. 28.—This staphylococcus was one of 12,972 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture: in litmus milk there is a strong acid production, coagulation, and a precipitate of casein; rapidly liquefies gelatine; in bouillon, cloudy with yellow precipitate; on agar, an orange moist glistening growth; on potato, a heavy yellow growth; in glucose bouillon, no fermentation, but cloudy all through; in lactose bouillon, no fermentation, cloudy all through.

Coccus No. 29.—This staphylococcus was one of 43 colonies which grew on our agar plates, planted from a tube of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, a strong acid production, coagulation, and precipitate of casein; very rapidly liquefies gelatine; bouillon slightly cloudy with yellow precipitate; on agar, an orange-yellow moist glistening growth; on potato, yellowish slowly spreading growth; in glucose bouillon, no fermentation, but growth all through; in lactose bouillon, no fermentation, but growth all through.

Coccus No. 30.—This staphylococcus was one of 81 colonies which grew on our agar plates, planted from a tube of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; acid production in litmus milk; liquefies gelatine very slowly; in bouillon, slightly cloudy, with yellow precipitate; on agar, a yellowish brown moist glistening growth; on potato, yellowish slowly spreading growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, but growth at bend of tube.

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	7
	14
	15
	17
	21
	22
	22
	31
	31 (Coc. 31.)
	53

This glycerinated virus was labeled by the manufacturer to be good until January 1, 1903, and was planted December 4, 1902.

The following organisms, resembling the pyogenic cocci, were isolated:

Coccus No. 31.—This staphylococcus was one of 31 colonies which grew on our agar plates planted from a tube of manufacturer No. 10.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, produces alkali with light yellow precipitate; does not liquefy gelatine; bouillon clear, with some yellow precipitate; on agar, a heavy yellow, shiny growth; on potato, a scanty yellow growth; in glucose bouillon, no fermentation, but slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

MANUFACTURER NO. 11.

Number of organisms found.	
Dry points.	Glycerinated virus.
283 (Coc. 32.)	
324	
333 (Coc. 33.)	
684	
728	
3,696	
9,072	
24,360 (Coc. 34.)	
29,145	
44,000	

These points had no date at all. They were planted November 19, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 32.—This staphylococcus was one of 283 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a slight acid production, coagulation, and a precipitate of casein; rapidly liquefies gelatine: in bouillon, slightly cloudy, with some yellow precipitate; on agar, orange yellow granular growth; on potato, yellow granular spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth all through tube.

Coccus No. 33.—This staphylococcus was one of 333 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; does not liquefy gelatine; in bouillon, slightly cloudy, with some white precipitate; on agar, a white shiny growth; on potato, nearly invisible white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, slight growth at bend of tube.

Coccus No. 34.—This staphylococcus was one of 24,360 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a strong acid production, coagulation, and precipitate of casein; slowly liquefies gelatine; bouillon is cloudy, with heavy white precipitate; on agar, a whitish yellow, shiny moist growth; on potato, yellow growth with white border; in glucose bouillon, no fermentation, growth all through tube; in lactose bouillon, no fermentation, growth all through.

SUMMARY OF SERIES III.

November and December, 1902.

Number of organisms found.				
Dry points.		Glycerinated virus.		
0	1,204	0	9	
0	1,399	0	10	
1	1,452	0	10	
1	1,800	1	11	
2	1,847	1	11	
3	1,946	2	11	
3	2,074	2	12	
3	2,244	2	13	
3	2,246	2	13	
3	2,584	2	14	
5	2,912	2	14	
5	3,696	2	15	
5	3,754	2	16	
6	5,952	2	16	
7	7,465	2	16	
11	7,632	3	17	
21	7,560	3	19	
31	7,980	3	19	
48	8,702	3	20	
67	8,748	3	21	
243	8,748	4	22	
262	9,072	4	22	
283	9,744	4	25	
324	10,022	4	31	
333	10,265	4	31	
420	10,982	4	31	
424	11,020	4	37	
452	12,923	4	41	
465	12,972	5	43	
475	15,552	5	47	
479	19,872	5	53	105
526	24,360	5	70	112
576	25,574	5	70	113
604	26,411	6	77	152
684	29,145	6	81	230
728	31,380	6	85	239
810	<u>44,000</u>	6	87	<u>236</u>
853		7	95	
868		7		
887		7		
1,088		7		
1,156		8		
1,199		9		
		9		
Number points examined.. 80		Number tubes examined..... 89		
Total bacteria..... 391,603		Total bacteria..... 2,592		
Average per point..... 4,895		Average per tube..... 29		

DRY POINTS AND GLYCERINATED VIRUS COMPARED FROM A BACTERIOLOGICAL STANDPOINT.

The question is often asked. Is glycerinated virus superior to dry points?

As the object of glycerinating the virus is to eliminate the foreign bacteria, the answer to this question resolves itself almost entirely into a comparative study of the number and kind of organisms found to contaminate this kind of vaccine.

The result of our studies on this point may be summarized as follows:

Series.	Average number of organisms.	
	Dry points.	Glycerinated virus.
Series I, winter, 1901-2	4,809	4,698
Series II, spring, 1902.....	3,458	1,058
Series III, winter, 1902-3	4,895	29

It is plain that last winter (Series I) the glycerinated virus contained a number of contaminating bacteria equal in amount to the dry points. This winter (Series III) shows a great improvement in the glycerinated virus and demonstrates conclusively that this form of vaccine may be, and should be freer from impurities than the dry points.

Of course the greater freedom from bacterial impurities of the glycerinated virus to the dry points refers only to those dry points made with fresh "lymph." There can be little choice between the dry points made with glycerinated pulp and the capillary tubes containing the same material.

The pyogenic cocci and other bacteria pathogenic for laboratory animals were found both in the glycerinated virus and on the dry points.

EXPERIMENTAL TETANUS IN VACCINE VIRUS.

Can tetanus be found in the vaccine virus bought in open market?

Many tubes containing glycerinated virus and many points of the dry vaccine were examined bacteriologically for tetanus, but we were unable to discover this organism.

This part of the work was done in several ways. Susceptible animals were inoculated and vaccinated with the contents of many tubes

and dry points. Sometimes the total contents of 10 or 12 tubes were injected subcutaneously into guinea pigs or mice. In another series the virus from ivory points or capillary tubes was planted in fresh broth and grown anaërobically, and the resulting growth studied for end-spore-forming rods and also inoculated into animals. In no case was an organism or were symptoms resembling tetanus found.

While these results were most gratifying, they must not be taken as conclusive that tetanus may not sometimes contaminate vaccine virus, although from our studies this must be exceedingly rare. Tetanus organism can not grow or produce its toxin either in the glycerinated virus or on the dry points. It would take gross carelessness to contaminate the vaccine with a sufficient number of tetanus spores to carry the disease to those vaccinated.

It is not a matter of surprise that some outbreaks of tetanus have occurred when thousands of open wounds are presented for the reception of this infection so widely distributed in nature. Local outbreaks or small epidemics of tetanus have been known before the days of antiseptics, due to causes so well understood nowadays, and it seems more likely that carelessness in the dressing or handling of the wound, the pernicious use of shields, or faulty technique in performing the operation has introduced the tetanus spores, than that they were contained in the vaccine virus.

Most of the following work on tetanus was done under my direction by Dr. Edward Francis, and it is a pleasure to acknowledge the care and faithfulness with which he carried out all the exacting details.

VIABILITY OF TETANUS IN VACCINE VIRUS.

Can tetanus live and grow in vaccine virus?

In order to answer this question we contaminated vaccinal serum and the glycerinated pulp with tetanus spores. The serum was dried upon ivory points and the glycerinated pulp was conserved in capillary tubes in a manner similar to the methods now used by the manufacturers. From time to time these points and tubes were examined.

For the purposes of these experiments it was thought best to use a pure culture of tetanus for one series of experiments and for another series to use a mixed culture composed of tetanus, a coccus, and a motile, spore-bearing, saprophytic rod, as representing more nearly the natural conditions under which tetanus would be found to gain entrance into vaccine.

The pure culture of tetanus used in these experiments was taken from our laboratory stock culture. It was planted into ordinary bouillon on March 2 and grown anaërobically in a Novy jar at 37° for seven days, when it contained abundant spores.

VIRULENCE OF THE TETANUS CULTURE USED.

In order to determine the virulence of the pure culture of tetanus used it was tested in white mice, with the following results. Each

quantity of pure bouillon culture indicated was inoculated into the right side of a mouse.

Mouse.	Quantity of tetanus culture injected on March 9.
	<i>c. c.</i>
1.....	0.000065
2.....	.000049
3.....	.000039
4.....	.000032
5.....	.000028
6.....	.000024
7.....	.000021

On March 10 the three mice which had received the largest doses showed typical symptoms of tetanus, while the four mice which had received the smallest doses showed no symptoms.

On March 11 the mouse which received the largest dose was dead. The second, third, fourth, fifth, and sixth showed typical symptoms of tetanus, while the seventh, which received the smallest dose, gave no symptoms whatever of tetanus.

On March 12 mice 2 and 3 were dead; 4, 5, 6, and 7 show typical tetanus.

On March 13 mice 4, 5, and 6 were dead; 7 shows typical tetanus.

On March 14 mouse 7 died.

Table showing virulence of the tetanus culture used in these experiments.

Mouse.	March 9.	March 10.	March 11.	March 12.	March 13.	March 14.
1	<i>c. c.</i> 0.000065	Typical tetanus.	Dead.			
2	.000049	do.....	Typical tetanus.	Dead.		
3	.000039	do.....	do.....	Do.		
4	.000032	Negative	do.....	Typical tetanus.	Dead.	
5	.000028	do.....	do.....	do.....	Do.	
6	.000024	do.....	do.....	do.....	Do.	
7	.000021	do.....	Negative	do.....	Typical tetanus.	Dead.

From these tests we considered 0.000021 *c. c.* to constitute about the minimum fatal dose of our culture for an average-sized white mouse.

VIABILITY OF A PURE CULTURE OF TETANUS IN GLYCERINATED VACCINE VIRUS.

The vaccine pulp was taken from the heifer on February 15 and subjected to the action of glycerin by the vaccine manufacturer for twenty-five days. It was then, on March 12, obtained by us and contaminated with our pure culture of tetanus in the following manner:

We aimed to imitate as closely as possible the ordinary conditions under which we find glycerinated vaccine on the market. So we drew out in the flame a large number of capillary tubes of the length and caliber ordinarily used by manufacturers to contain material sufficient for one vaccination. Definitely measured amounts of the pure culture of tetanus were added to large drops of the glycerinated virus, and this mixture was drawn up into the small capillary tubes, which were hermetically sealed in the usual manner. In this way we had an accurate gauge of the exact amount of tetanus put into each capillary tube.

These tubes were filled with the glycerinated virus and tetanus spores on March 12. Each capillary tube contained one drop of glycerinated virus plus the following amounts of tetanus culture:

[.000021=MLD, minimal lethal dose.]

First series	=0.000055 c. c. (about 2½ MLD).
Second series	.00011 c. c. (about 5 MLD).
Third series	.00022 c. c. (about 10 MLD).
Fourth series	.00040 c. c. (about 20 MLD).
Fifth series	.00074 c. c. (about 40 MLD).
Sixth series	.013 c. c. (about 500 MLD).

The capillary tubes were tested immediately in mice as a control and then placed in the cool chamber at 20°-22° C., protected from light, and again tested at intervals in mice and in cultures, as indicated in the following tables:

Capillary tubes containing 0.000055 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

[P=positive, i. e., symptoms of tetanus; N=negative, i. e., no symptoms; †=death.]

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.								Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	
Mar. 12	Mar. 12	0	P	P	P	P	P	†			Positive; death.
Do.....	Apr. 11	30	P	P	†						Do.
Do.....	May 21	70	P	P	P	P	†				Do.
Do.....	June 17	97	N	P	P	P	P	P	P	†	Do.
Do.....	July 19	129	N	P	P						Positive; recovered.
Do.....	Aug. 1	142	N	N	P	P	P				Do.
Do.....	Aug. 28	169	N								Negative.
Do.....	Sept. 3	175	N								Do.

We see from this series that the tetanus spores neither multiplied nor produced toxin. In fact, they gradually lost virulence. Each capillary tube in this series of experiments contained approximately two and a half times the minimal lethal dose of the tetanus culture. It

will be noted that the virulence began to diminish noticeably at the fourth month and failed to produce tetanus after the lapse of five months.

Capillary tubes containing 0.00011 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12 ..	June 17	97	N	P	P	†	.	.	.	.	.	.	Positive; death.
Do...	Aug. 28	169	N	N	N	N	.	.	.	.	.	.	Negative.
Do...	Sept. 3	175	N	N	N	N	.	.	.	.	.	.	Do.
Do...	Sept. 9	181	N	N	N	N	.	.	.	.	.	.	Do.

The series contained approximately five times the minimal fatal dose of tetanus. It was first tested after the lapse of three months, when it was still virulent; but after seven months it had entirely lost its power of producing the disease when inoculated directly into mice. Evidently the tetanus spores did not germinate or produce toxin.

Capillary tubes containing 0.00022 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

[illegible]

This series was contaminated with ten times the minimal fatal dose. In this large quantity the spores maintained their toxicity a very long time—two hundred and forty-eight days—although they failed to produce the disease after ten months.

Capillary tubes containing 0.00040 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

[illegible]

Each capillary tube in this series contained about twenty times the minimal fatal dose of tetanus, which lost its virulence after ten months.

Capillary tubes containing 0.00074 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	P	P	†								Positive; death.
Do...	Oct. 1	203	P	P	P	P	P	P	P	P	P	†	Do.
Do...	Nov. 15	248	P	P	†								Do.
Do...	Jan. 1	295	P	P	†								Do.
Do...	Feb. 1	326	N	P	†								Do.
Do...	Mar. 2	355	N	P	†								Do.

It will be seen that this large amount of tetanus (about forty times the minimal fatal dose) remained alive and virulent in glycerinated virus one year.

Capillary tubes containing 0.013 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	P	†									Positive; death.
Do...	Oct. 1	203	P	†									Do.
Do...	Nov. 15	248	P	†									Do.
Do...	Jan. 1	295	P	†									Do.
Do...	Feb. 1	326	P	†									Do.
Do...	Mar. 2	355	N	P	†								Do.

This large amount (about $500 \times \text{MLD}$) has also remained alive and virulent for mice one year. The material is not yet exhausted, and will be tested from time to time.

VIABILITY OF PURE CULTURE OF TETANUS ON DRY POINTS.

The serum which exuded from the site of the inoculation after the superficial layers of the variolous eruption was removed was obtained from a calf on March 12, 1902. This serum, commonly called "lymph," when dipped or brushed upon the ivory points and allowed to dry, formerly constituted the "dry points" of the manufacturers. At present some dry points are made with glycerinated virus alone or with glycerinated pulp mixed with blood serum and in other ways. We, however, preferred to use the "lymph" or serum, as it seemed to

offer a more favorable medium for preserving the vitality of the tetanus spores.

The sterile ivory points were prepared in the usual manner with the vaccinal serum but contaminated with definite measured amounts of tetanus culture.

Each point of the first series contained 0.00012 c. c. of pure bouillon culture of tetanus; each point of the second series contained 0.0002 c. c. of pure bouillon culture of tetanus; each point of the third series contained 0.0004 c. c. of pure bouillon culture of tetanus; each point of the fourth series contained 0.001 c. c. of pure bouillon culture of tetanus.

These were allowed to dry in the usual manner and kept in the cool chamber at 20°–22° C., well protected from the light. These points were tested at intervals on mice and in cultures as shown in the following tables:

Dry points containing vaccine serum ("lymph") were contaminated with 0.00012 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	May 28	77	N										Negative.
Do.....	June 17	107	N										Do.
Do.....	June 27	117	N										Do.

It will be seen that a quantity of tetanus spores equal to five times the minimal lethal dose lost its toxicity for mice within seventy-seven days. The spores, however, were not dead, for these points planted in bouillon and grown anaerobically in a Novy jar for one week gave a culture rich in end spore-bearing rods, which caused typical tetanus in a mouse in doses of 0.001 c. c.

Dry points containing vaccine serum ("lymph") were contaminated with 0.0002 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12 ..	May 28 .	77	N										Negative.
Do.....	June 17.	107	N										Do.
Do.....	June 27.	117	N										Do.

These points, containing approximately ten times the minimal fatal dose of tetanus, apparently soon lost their virulence when inoculated

directly into mice. But when grown anaerobically, as above, the spores multiplied and regained their pathogenic power.

Dry points containing vaccine serum ("lymph") were contaminated with 0.0004 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st. day.	2d. day.	3d. day.	4th. day.	5th. day.	6th. day.	7th. day.	8th. day.	9th. day.	10th. day.	
Mar. 12	May 28	77	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do...	June 17	107	N										Negative.
Do...	June 27	117	N										Do..

This series of dry points, contaminated with about forty times the minimal dose of tetanus, remained virulent over seventy-seven days, but failed to produce symptoms after one hundred and seven days. The spores, however, were not dead; for, planted in bouillon and grown in a Novy jar for seven days, they gave a rich culture, which produced typical symptoms of tetanus in a mouse.

Dry points containing vaccine serum ("lymph") were contaminated with 0.001 c. c. of tetanus culture. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st. day.	2d. day.	3d. day.	4th. day.	5th. day.	6th. day.	7th. day.	8th. day.	9th. day.	10th. day.	
Mar. 12 ..	May 28	77	P	†									Positive; death.
Do.....	June 27	107	P	P	†								Do.
Do.....	Aug. 1	142	?	P	P	P	P	†					Do.
Do.....	Aug. 28	169	N	P	†								Do.
Do.....	Sept. 30	202	N	P									Positive.
Do.....	Nov. 15	248	P	P	P	P	P	P	P	P	P	†	Positive; death.
Do.....	Jan. 1	295	N	N	P	P	P	P	P	P	†		Do.
Do.....	Feb. 1	336	N										Negative.

The large amount of tetanus in this series of points remained virulent, when directly inoculated into mice, for a period of two hundred and ninety-five days. This shows well the long life of dried tetanus spores when kept in a cool place, protected from the light—that is, under the conditions under which vaccine virus is usually conserved.

VIABILITY OF TETANUS IN MIXED CULTURE IN GLYCERINATED VIRUS.

In the following experiments we used very small quantities of a culture of tetanus mixed with other organisms, viz, a coccus and a saprophytic motile rod with a central spore. This mixture was planted into ordinary bouillon April 1 and incubated at 37° C. in a Novy jar

for two weeks. The resulting growth was then tested on mice to determine its virulence. Each of two mice were given 0.00002 of the culture. Both animals took the disease. The one died in eight days; the other recovered. One month later, viz, May 28, two more mice were inoculated with an equal amount of the same culture, and both died. This amount was, therefore, considered to represent approximately the minimal fatal dose, and was used to contaminate the vaccine virus.

The glycerinated virus of two manufacturers was bought in the open market. With the mixed tetanus culture we prepared an artificial contamination of both these viruses and sealed them hermetically in small capillary tubes. By means of dilution each capillary tube was made to contain just 0.00002 c. c. of the tetanus culture in addition to a large-sized drop of the glycerinated virus.

These tubes were kept at 20°-22° C. in a dark place and tested from time to time.

Glycerinated virus (manufacturer No. A) contaminated with 0.00002 c. c. of a mixed culture of tetanus.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Apr. 30	Apr. 30	0	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do..	May 28	28	N										Negative.
Do..	June 17	48	N										Do.
Do..	June 27	58	N										Do.

We see from this table that one month's action of the glycerinated virus reduced the virulence of the tetanus which it contained to a point where it no longer produced any symptoms on mice.

A comparison of the table above with the control which immediately precedes it indicates strikingly the attenuating power of the glycerinated virus in this instance. Observe that on May 28 the glycerinated tube produced no symptoms of tetanus, while the two control tests made on the same date both proved typical tetanus. Whether the virus had killed the tetanus or merely diminished its virulence was determined by planting on July 8 six of the tubes each into a separate cubic centimeter of bouillon and growing it under anaërobic conditions for one week. At the end of this time we tested the growth in mice and were surprised to find that three of the tubes when injected into mice caused no sickness whatever, while the other three tubes produced typical symptoms of tetanus in mice.

We conclude that the glycerinated virus killed the tetanus entirely in some of the tubes in two months and that if it had been allowed to act longer on the other tubes they, too, probably would have contained no living tetanus.

Glycerinated virus (manufacturer No. B) contaminated with 0.0000? c. c. of a mixed culture of tetanus.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Apr. 30..	Apr. 30	0	P	P	P	P	(†)						Positive; death.
Do.....	May 28	28	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do.....	June 17	48	N	P	P	P	P	P	P	(†)			Positive; death.
Do.....	June 27	58	N	P	P	P	P	P	P	P	P	(†)	Do.
Do.....	do....	58	N	N	N	P	P	P	P	P	P	P	Positive; recovered.
Do.....	July 18	79	N	N	P	P	P	P	P	P	P	P	Do.
Do.....	Aug. 1	93	N	N	P	P	P	P	P	P	P	P	Do.
Do.....	Aug. 28	120	N										Negative.
Do.....	Sept. 3	126	N										Do.
Do.....	Sept. 6	129	N										Do.
Do.....	Sept. 29	152	N										Do.

This table shows that after August 28 the tubes failed to produce tetanus in the mice. Therefore, on September 30, we subjected the remaining two tubes to cultivation in bouillon for one week and found that in one of the tubes the tetanus was still alive, for the growth showed rods bearing end spores and produced typical tetanus in mice.

In the other tubes there was no growth of tetanus.

A comparison of these two tables shows that make No. B of glycerinated virus required four months to attenuate the same amount of tetanus which make No. A attenuated in one month and killed completely in two months. This fact led to a study of the comparative effects of the different percentages of pure glycerin on the tetanus organism and its toxin. This study will be reported upon later.

VIABILITY OF TETANUS IN MIXED CULTURE ON DRY POINTS.

The same mixed culture of tetanus used in the two preceding experiments was also used to contaminate dry points. The tips of the sterilized ivory points were dipped into vaccine serum freshly obtained from the calf. To each point was then immediately added a definite measured amount of the tetanus culture. The points were allowed to dry in the air and kept in a cool (20° – 22° C.), dark place until tested.

To contaminate the first series 0.001 c. c. of the tetanus culture was used; 0.0004 c. c. of the tetanus culture was used to contaminate the second series; 0.0002 c. c. of the tetanus culture was used to contaminate the third series; 0.00012 c. c. of the tetanus culture was used to contaminate the fourth series.

Series of dry points containing various amounts of mixed culture of tetanus, prepared June 2.

Amount of tetanus culture.	Inoculated into mice.	Result.
c. c.		
0.001	June 17.....	Remained negative.
	June 27.....	Do.
.0004	June 17.....	Do.
	June 27.....	Do.
.0002	June 17.....	Do.
	June 27.....	Do.
.00012	June 17.....	Do.
	June 27.....	Do.

Compare this table with the control experiments on page 28 and we see the striking attenuating effect of exposure of tetanus on dry points.

For instance: On May 28 two tests of the control culture killed mice with typical symptoms of tetanus; whereas, on June 17, after an exposure of two weeks on dry points, doses of tetanus which represented as much as fifty times the control doses had been rendered nontoxic for mice. It now remained to determine whether the tetanus on the point was dead or only attenuated. It was found after growing a point of each series in 1 c. c. of bouillon for one week that the growth showed end-spore bearing organisms in microscopic preparations and produced typical symptoms of tetanus when inoculated into mice.

Again, on October 4, four months after the dry points were prepared, specimens from series 0.0004, 0.0002, and 0.00012 c. c. were planted into bouillon and grown anaërobically. The culture again contained end spore bearing organisms which, inoculated into mice, produced typical symptoms of tetanus. The tetanus spores on the points were therefore not dead, although they had lost their virulence for mice when inoculated directly.

SUMMARY AND CONCLUSIONS.

We examined the vaccines of 10 different manufacturers during a period of more than a year.

All the samples examined were purchased on the open market, care being taken to buy unbroken original packages from reliable pharmacists who keep the product under proper conditions of light and temperature. With a few exceptions, the vaccine was examined before the time limit expired, if the time was given by the manufacturer.

Of 190 dry points examined we found an average of 4,354 bacteria per point. A number of these points contained over 15,000 and one as high as 44,000 organisms.

Of 244 tubes of glycerinated virus examined we found an average of 1,742 bacteria per tube. A number of these capillary tubes contained over 10,000 bacteria and one as high as 30,000. This is of course much more than a carefully prepared glycerinated virus should contain.

We found the pus cocci and other bacteria, pathogenic for laboratory animals, in both the dry points and the glycerinated virus.

We have demonstrated that some of the glycerinated virus marketed during the winter of 1901-2 contained an excessive number of bacteria, which decreased notably after a few weeks, indicating the sale of a "green" or unripe product; that is to say, the virus was not glycerinated a sufficient length of time before it was sold.

It was also plain from our studies that too much confidence was placed by the producers in the germicidal power of glycerin.

The excessive impurities found in some of the glycerinated virus upon the market we believe was largely due to this overconfidence in the germicidal value of glycerin; operators become careless, trusting to the glycerin to purify their product. We know that glycerin is too feeble in its properties to purify vaccine matter which has initial contamination such as our work indicates.

After we called attention to this point we found a marked improvement in the glycerinated virus on the market. For instance:

During the winter of 1901-2 the glycerinated virus contained an average of 4,698 bacteria per tube.

In the spring (April and May) of 1902 the average fell to 1,058 bacteria per tube.

This winter (November and December), 1902, the average of 89 tubes examined was only 29 bacteria—maximum 239.

Glycerinated virus, when properly prepared and kept a sufficient length of time, is freer from impurities than dry points made with fresh "lymph."

There is practically no difference between the glycerinated virus dried upon ivory points and that hermetically sealed in capillary tubes, so far as bacteriological impurities are concerned.

It will be seen that there is practically no vaccine on the market free from bacterial contamination, although the product of some manufacturers is remarkably pure.

The unevenness of the purity of vaccine as marketed by some manufacturers is very marked. The greater contamination of the vaccine during the winter of 1901-2 may be accounted for, in part, by the undue haste and accompanying carelessness incidental to supplying the increased demand for vaccine virus at that period.

We have examined a great number of dry points and the contents of glycerinated tubes for tetanus, but have been unable to find the organism of this disease.

Tetanus spores may live a long time in vaccine virus. We have found them alive and virulent on dry points after two hundred and ninety-five days and in glycerinated virus sealed in capillary tubes three hundred and fifty-five days.

Tetanus may become a contaminating element of vaccine before it leaves the heifer. During the period of three to five days which elapses between the vaccination of the heifer and the removal of the virus there is opportunity for tetanus to find a lodgment in the eruption on the heifer's body surface, provided tetanus is present in the stall or stable surroundings of the animal.

If tetanus reaches the heifer's vaccinated area it may contaminate both the "dry points," which are made directly from the "lymph," and also the vaccine pulp, which the manufacturer subjects for a longer or shorter time to the germicidal action of glycerin before he markets it as "glycerinated virus."

Tetanus added to glycerinated vaccine virus does not germinate when kept hermetically sealed and under anaërobic conditions in small capillary tubes. It gradually loses its virulence both in the tubes and on the ivory points. Although the virulence is lost, the tetanus spores are not necessarily dead; for, while they will not produce symptoms when inoculated directly into mice, they may be revived into active virulent cultures by growing in fresh bouillon under favorable conditions. That is to say, the vegetability of the spore remains active long after it has lost its power to produce the disease when inoculated directly into mice.

Therefore, in looking for tetanus in vaccine virus it is best to make cultures first and study the growth for end spore-bearing rods and then test the effects of the culture in animals.

The spores of tetanus lose their virulence and die much more quickly in the vaccine lymph on dry points than in the glycerinated tubes.

The vitality of tetanus in glycerinated virus depends largely upon the number of spores contaminating the virus. Large quantities, namely, forty times the minimal lethal dose ($40 \times \text{MLD}$), remain alive and active over a year. Smaller amounts may disappear in four to seven months. Very small amounts ($1 \times \text{MLD}$) have in one of our experiments lost virulence in one month and failed to grow in bouillon in two months.

On the other hand, these very small amounts of tetanus may remain active for months in glycerinated virus in capillary tubes. In one instance as small a quantity as 0.000055 c. c., which was about two and a half times the minimal fatal dose, remained active seven months.

In vaccinal "lymph" on dry points the spores may begin to lose their virulence in two weeks and be dead in two months. Usually they live longer. We found them alive and virulent in points that had been contaminated with $50 \times \text{MLD}$ about ten months (two hundred and ninety-five days).





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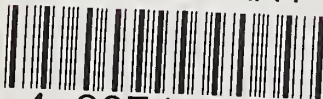


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